

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Group Art Unit: Thai-An N. Ton

Kangsheng Wang

Examiner: 1632

Serial No. 09/781,046

Filed: February 8, 2001

For: A Method and System for
Introducing a Gene into a Human Stem
Cell

AFFIDAVIT OF KIRON KANGSHENG WANG

PURSUANT TO 37 C.F.R. § 1.132

APPENDIX A

Linker Based Sperm-Mediated Gene Transfer Technology

1. Over-Immunization of Balb/C Mice with Mouse Sperm Cells
2. Screen Hybridomas Which Does Not Prevent Sperm Fertilization by In Vitro Fertilization
3. Flow Cytometry Analysis of mAbs Bound to Mouse Sperm Cells
4. Generation of Transgenic Mice from Two Different Linkers mAb C and mAb D

Over-Immunization of Balb/C Mice with Mouse Sperm Cells

TITLE



PROJECT NO.

BOOK NO.

1

Work continued from Page

1. Immunize 3 Balb/c mice with 2×10^6 ~~B6D2F1~~ B6D2F1 and FVB male sperm
overimmune 8 time (twice/month) (2 for FVB sperm)
1 for B6D2F1 sperm)

a. dissect epididymis of 12-15 weeks olds male, squeeze the sperm
out from and let sperm in Modified Tyrode's medium without
BSA.

b. wash sperm with MTM three time' and immunize mix with 200ul
TDM. count Number

c. Immunize 5 weeks old Balb/c female. (twice/month)

SCIENTIFIC BINDERY PRODUCTIONS CHICAGO 60605 MADE IN USA

Work continued to Page

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Kangsheng Wang

DATE 3/15/99

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DATE 10-1-99 B 100 P ©

Screen Hybridomas Which Does Not Prevent Fertilization
by In Vitro Fertilization

(screening assay shows that a number of hybridomas
supernatants does not inhibit sperm cells bound with
antibodies in the supernatant to fertilize oocytes.)

Work continued from Page

Test in vitro fertilization efficiency and blocking by

mAb

(hybridoma supernatant)

1. Set 20 egg/assay for IVF study, and

2. inject ~~with~~ 5 I.U. PMS (Sigma) ~~to~~ in 8 p.m. (day 1)50 B6D₂F₁ female with
8 weeks old

was

3. 48 hours later, each mouse injected with 5 I.U. hCG (day 3)

4. On day 4 at 7:30 a.m., the sacrifice the female mice and
collect egg from with cumulus cell from swollen ampulla
in MTM medium.5. ~~Add~~ Distribute each one clump of cumulus cell with egg to ^{(average 20}
each ~~is~~ ^{well} of 48 well dish in 200 μ l MTM medium ^{eg.}6. Add 20 μ l of supernatant of hybridoma to each well and
incubate with 5×10^4 sperm in 30 μ l MTM medium for 30 min7. add sperm mix to (5) and incubate in 37°C for
in (6) 4 hr for in vitro fertilization8. ~~collect~~ collect and transfer ^{fertilized} eggs to CZB medium and incubate
at 37°C for 20-22 hrs in ~~96~~ ¹⁰⁰ well9. observe the fertilization efficiency (++) ^{No} block fertilization ~~very~~ ^{of sup. of}
(++) ^{hybridoma}
(++) block ~~of~~ some
(+) block was a little
blank blocked
Work continued to Page

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Ken Wang

DATE

7/10/99

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10-1-99

Supernatant from
hybridoma from mouse
immunized with
FVB and B6D₂F₁ mouse
sperm

Group 3 (+)

1 A 4
1 B 5
1 F 6
2 A 10
2 B 2
2 E 3
2 F 4
3 B 7
3 D 4
4 C 8
4 C 9

Group 2 (++)

3 H 6
4 A 8
4 B 4
4 E 3
4 E 8
(16)

Group 1 (+++ or +++)

1 B 3
1 F 5
2 D 4
2 E 8
3 C 7
4 E 7
(6)

1	2	3	4	5	6	7	8	9	10	11	12
A1	A2	A4	A8	A10	B3	B4	B5	B6	B8	B9	C2
++	++	+	++	++	+++	++	+	++	++	++	++
C4	C7	C8	C9	D4	D6	E4	E5	E6	E12	F4	F5
++	++	++	++	++	++	++	++	++	++	++	++
F6	F12	G2	G5	G8	G9	G10	H1	H5	H10	(A2)	A7
++	++	++	++	++	++	++	++	++	++	++	++
A10	A12	B1	B2	B3	B6	B7	B11	C3	C4	C5	C6
++	++	++	++	++	++	++	++	++	++	++	++
G8	G10	C11	D4	E2	E3	E4	E6	E7	E8	E9	F4
++	++	++	++	++	++	++	++	++	++	++	++
F5	F9	F10	F4	G7	G12	H7	H8	H10	(A3)	A1	A3
++	++	++	++	++	++	++	++	++	++	++	++
A7	A10	B1	B3	B7	B8	C3	C5	C7	C10	C11	D3
++	++	++	++	++	++	++	++	++	++	++	++
D4	D8	D10	E5	E1	E10	E3	F5	F6	F10	(A2)	G3
++	++	++	++	++	++	++	++	++	++	++	++

1	2	3	4	5	6	7	8	9	10	11	12
A6	G6	H2	H6	H9	(A4)	A2	A8	B2	B7	B6	B7
++	++	++	++	++	++	++	++	++	++	++	++
B12	C1	C7	C8	C9	D1	D5	D8	D10	D12	E2	E3
++	++	++	++	++	++	++	++	++	++	++	++
E5	E6	E7	E8	E9	E10	F1	F2	F3	F4	F6	F9
++	++	++	++	++	++	++	++	++	++	++	++
F10	F11	G3	G5	G6	G7	G10	NC				
++	++	++	++	++	++	++	++	++	++	++	++

Retest

group 3 (+)

group 2 (++)

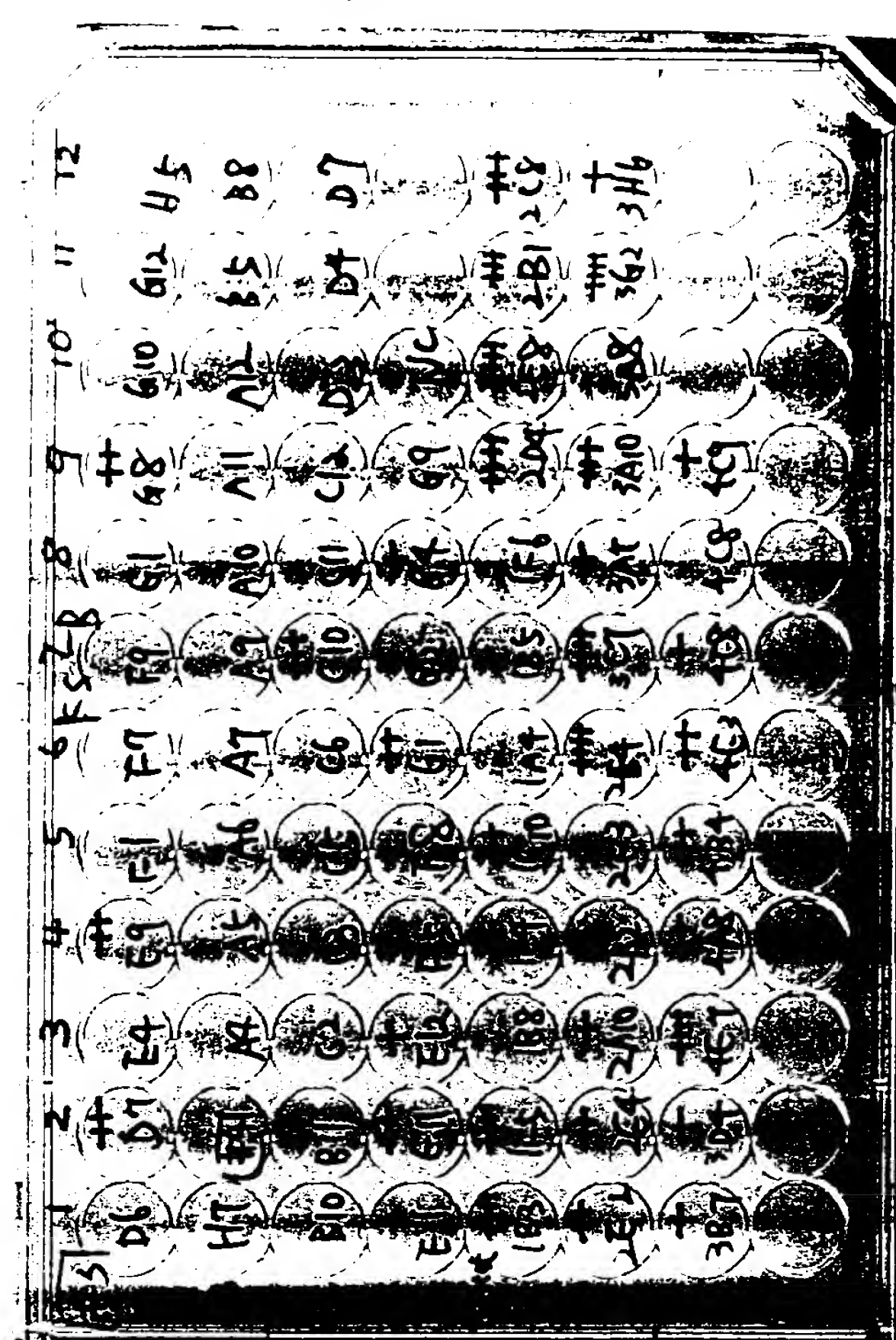
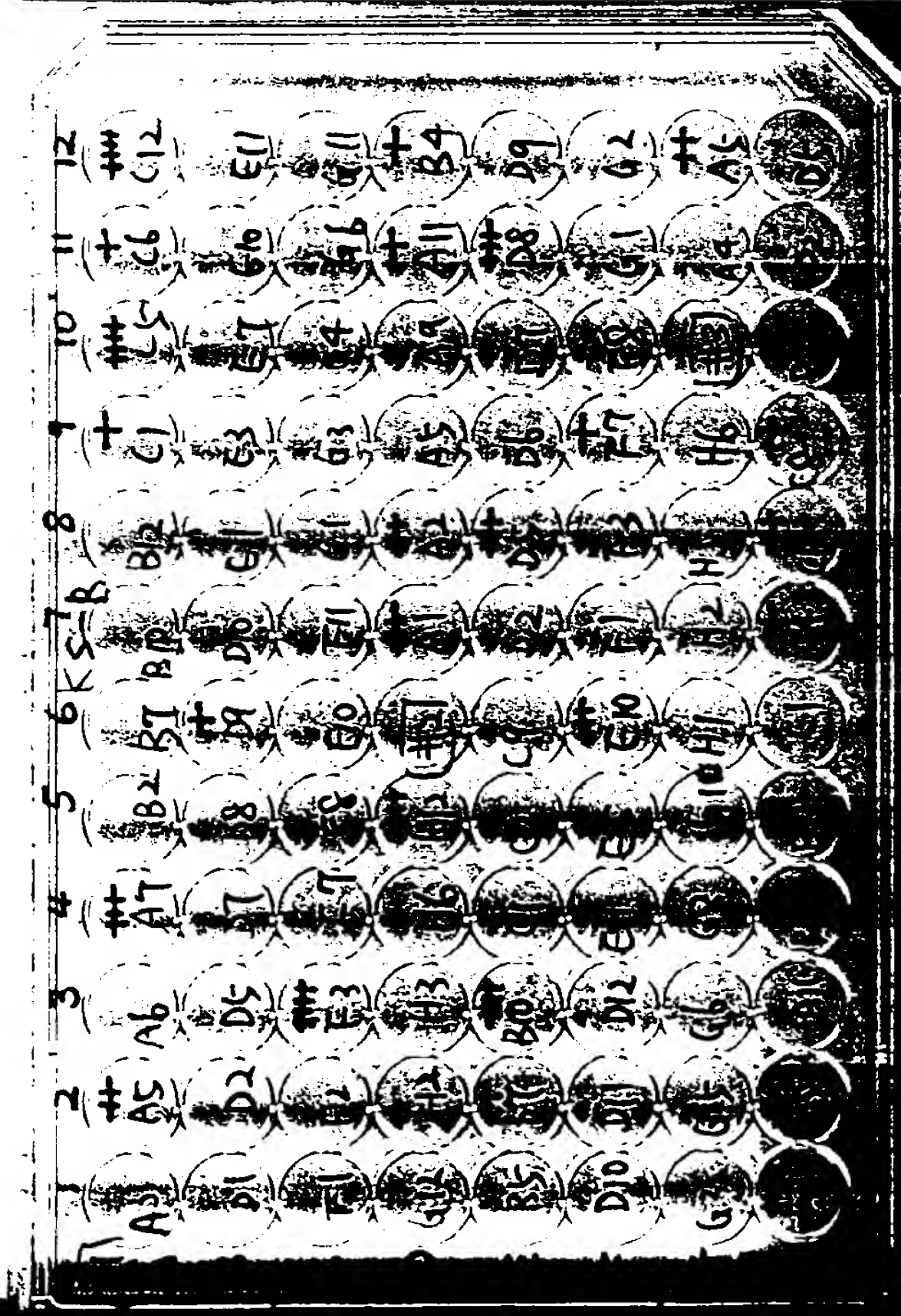
group 1 (+++ or +++)

Two-time
① show (+++ or +++) show ++ once
two-time twice

1 A 7	1 A 5	1 C 1
1 C 5	2 A 1	0 C 6
1 C 12	2 A 2	1 D 9
1 F 3	3 A 5	2 A 11
1 H 12	3 C 6	2 B 4
2 B 10	3 C 8	2 B 7
2 D 5	3 D 7	2 B 7
2 D 8	3 E 9	3 C 4
2 E 10	3 G 8	4 E 12
3 A 6	4 E 11	4 G 4
4 C 10		

2 B 1
2 C 8
3 A 10
3 G 2
2 E 8
3 C 7
4 E 7
1 G 10
2 E 2
2 E 4
4 A 8
4 B 4
4 E 3

③ show ++ twice



183

2E7 (##) twice

2D4

✓ 2C3 (++) Once

307

2D10 (+) once (+) once

2E8

1C10 (tt) twice

4E7

2E8 (TT) twice

155

$$v_{IH4}(t) \text{ once}$$

7B6 (+) twice (0) once

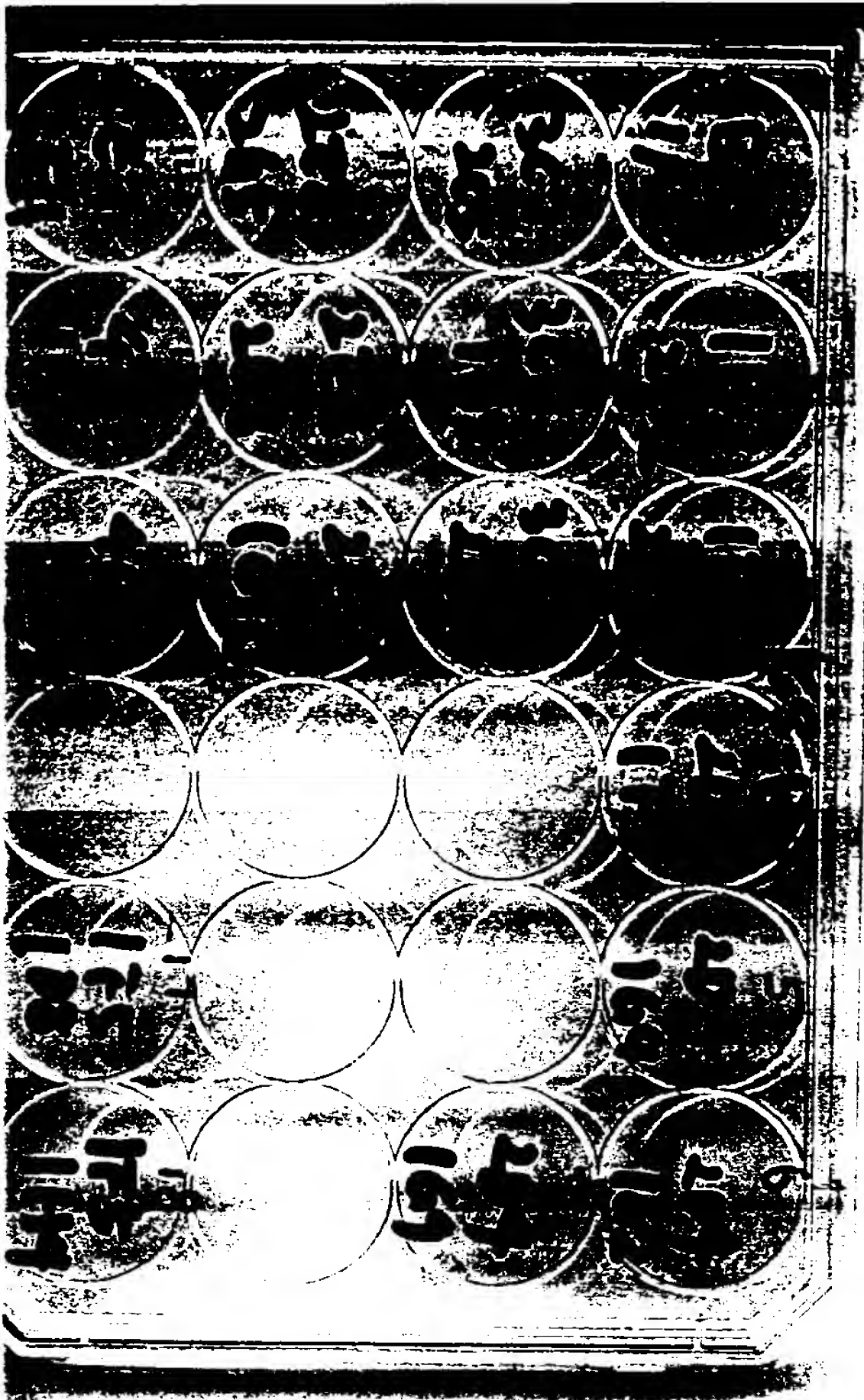
TITLE

PROJECT NO.

BOOK NO.

37

Worl



B group do isotype assay, we got $I_{\gamma M}$ all

so we select I_{B3} 1A8 (A) to do ascite fluid

I_{B3} 1A8 (B)

183	1A8 (A)
204	1F3 (B)
3C7	2C5 (C)
2E8	2F5 (D)
4E7	1F11 (E)
1F5	1D8 (F)

SCIENTIFIC BUREAU PRODUCTIONS, INC. 2435 17th Ave. N.W.

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10-1-99

Work continued to Page

Flow Cytometry Analysis of mAbs Bound to Mouse Sperm Cells

(Four mAbs A, B, C and D show the binding of mouse sperm cells)

9/2

Cow

BAG (Ken)
FITC

2 Sep 99
1530-1645

HdN
488(I20)/500nmW

cattle	L1	P1	Log	PM1	N. END
90	L1	P2	Log	580V	PM1 1
ITC	L2	P3	Log	620V	3
Auto	L2	M	Log	550V	5

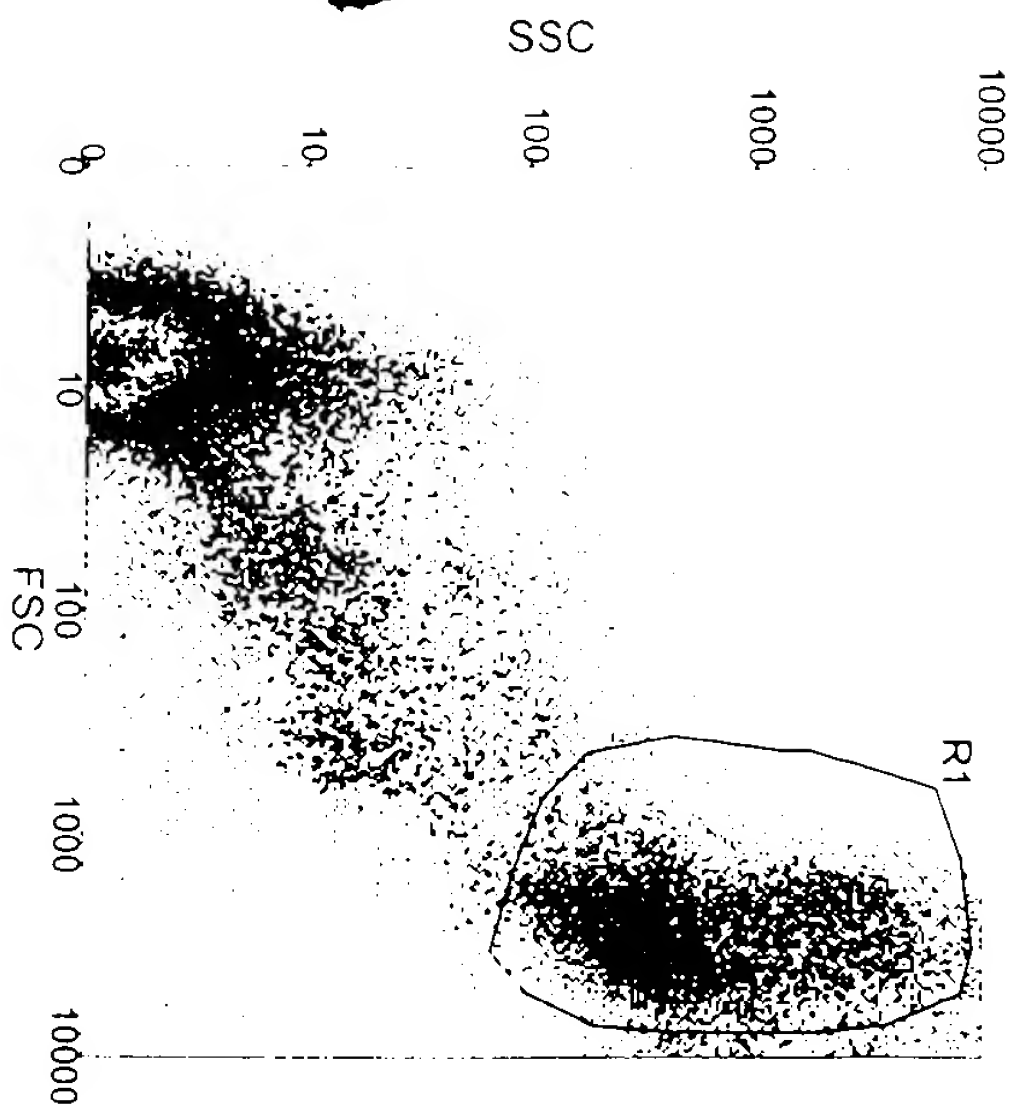
No Threshold

60.5/60.0

BAG-1999-09-02-000	Bovine	Extender	Auto
-001	Bovine	Extender	2 nd only
-002	"	"	Myelin
-003	"	"	C
-004	"	"	D
-005	"	PBS	Auto
-006	"	"	2 nd only
-007	"	"	Myelin
-008	"	"	C
-009	"	"	D

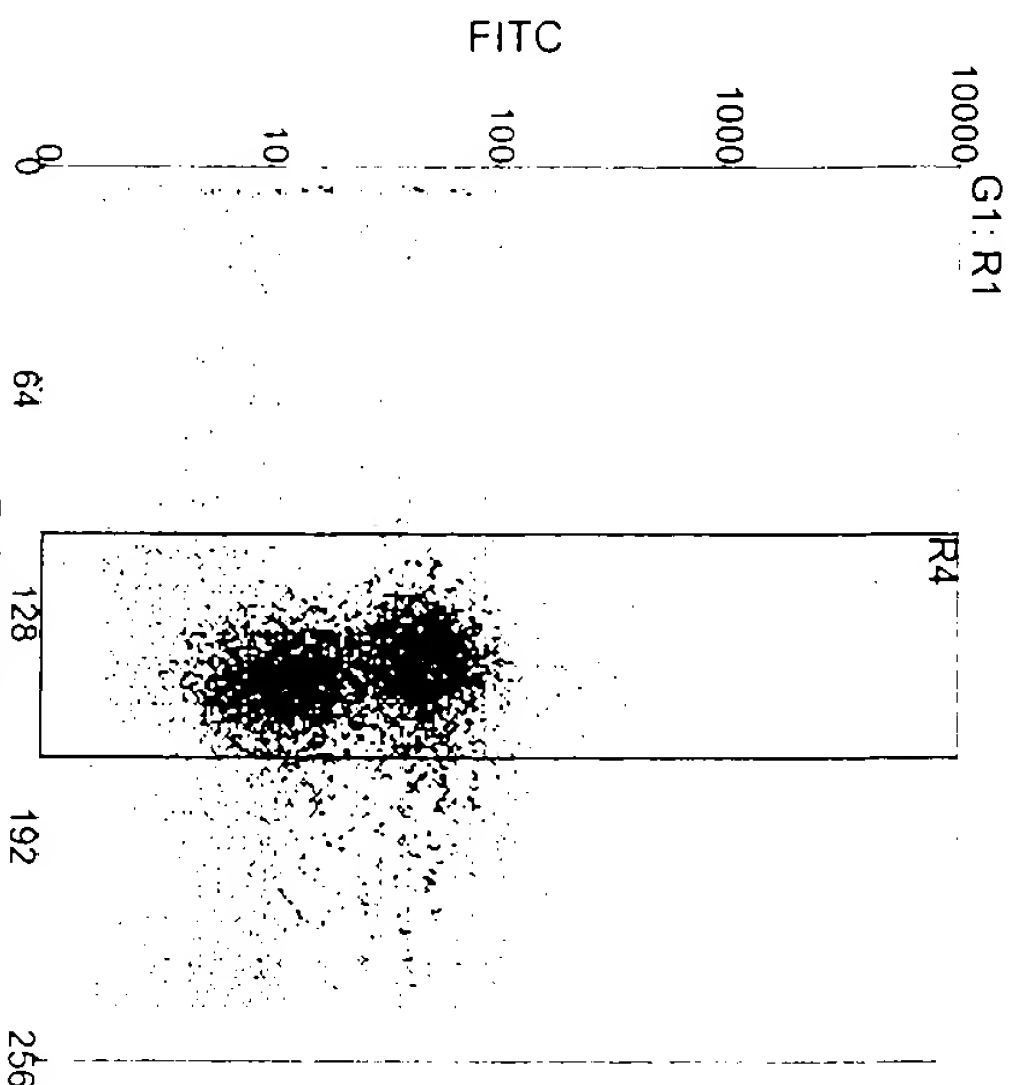
Bovine Sperm #4

-010	Mouse	Auto
-011	"	2 nd only
-012	"	Myelin Tubulin
-013	"	Myelin
-014	"	A
-015	"	B
-016	"	C
-017	"	D

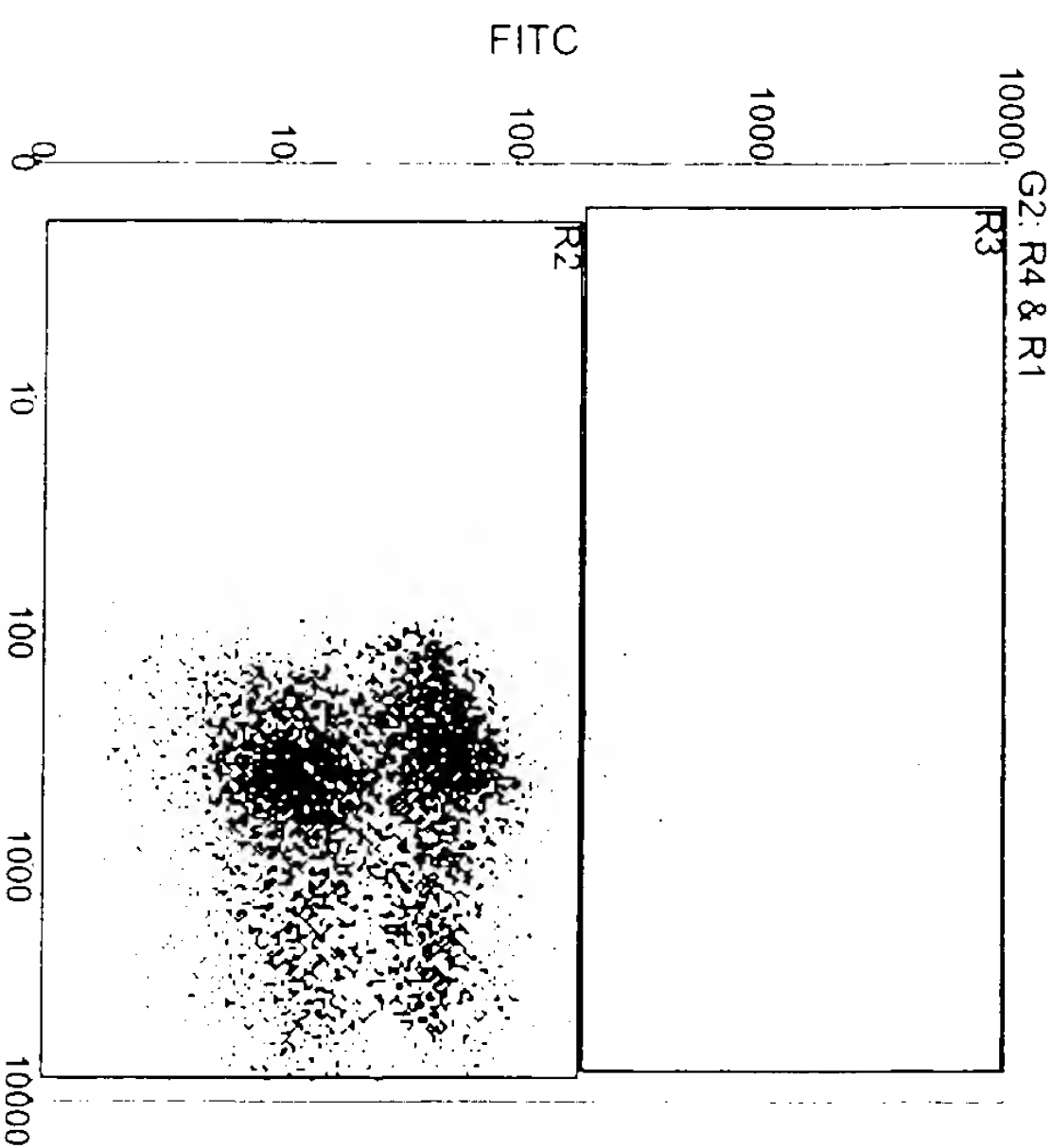


Region	Count	%	Mean
Total	50000	100.00	598.87, 192.30
R1	8385	16.77	2853.56, 898.98

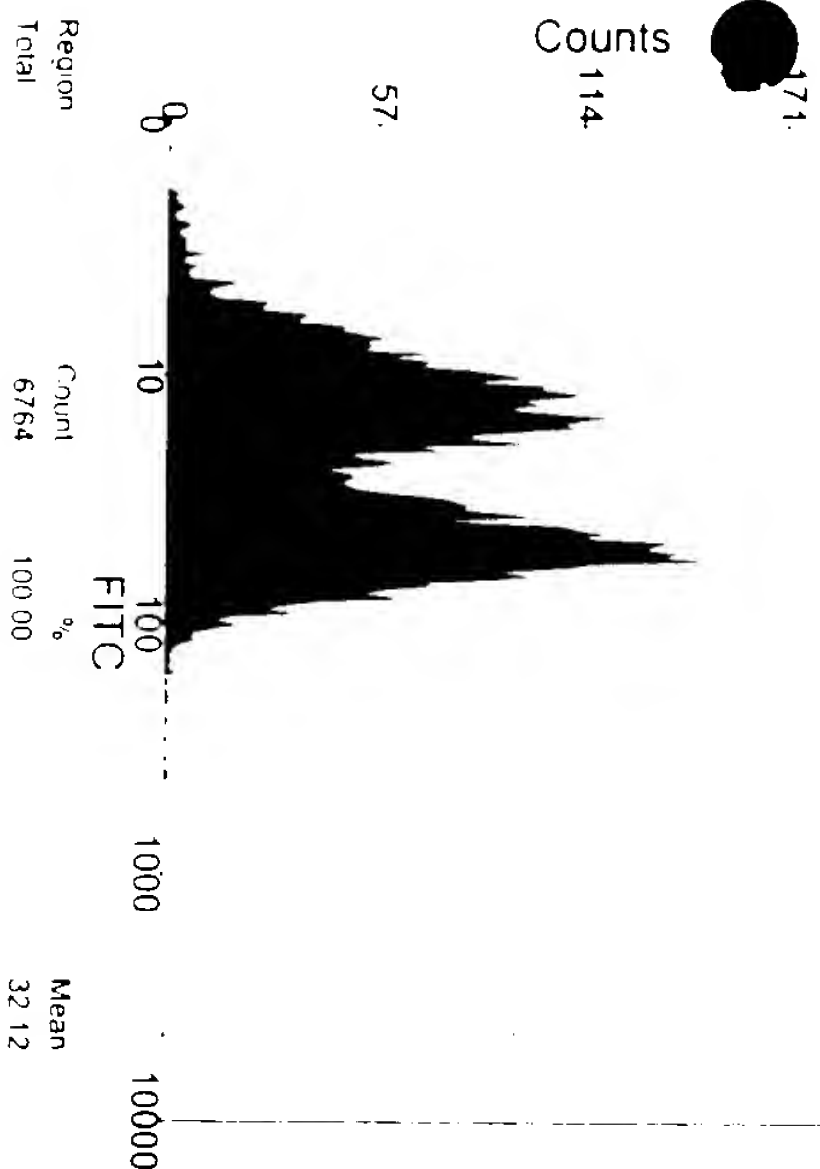
229 G2: R4 & R1



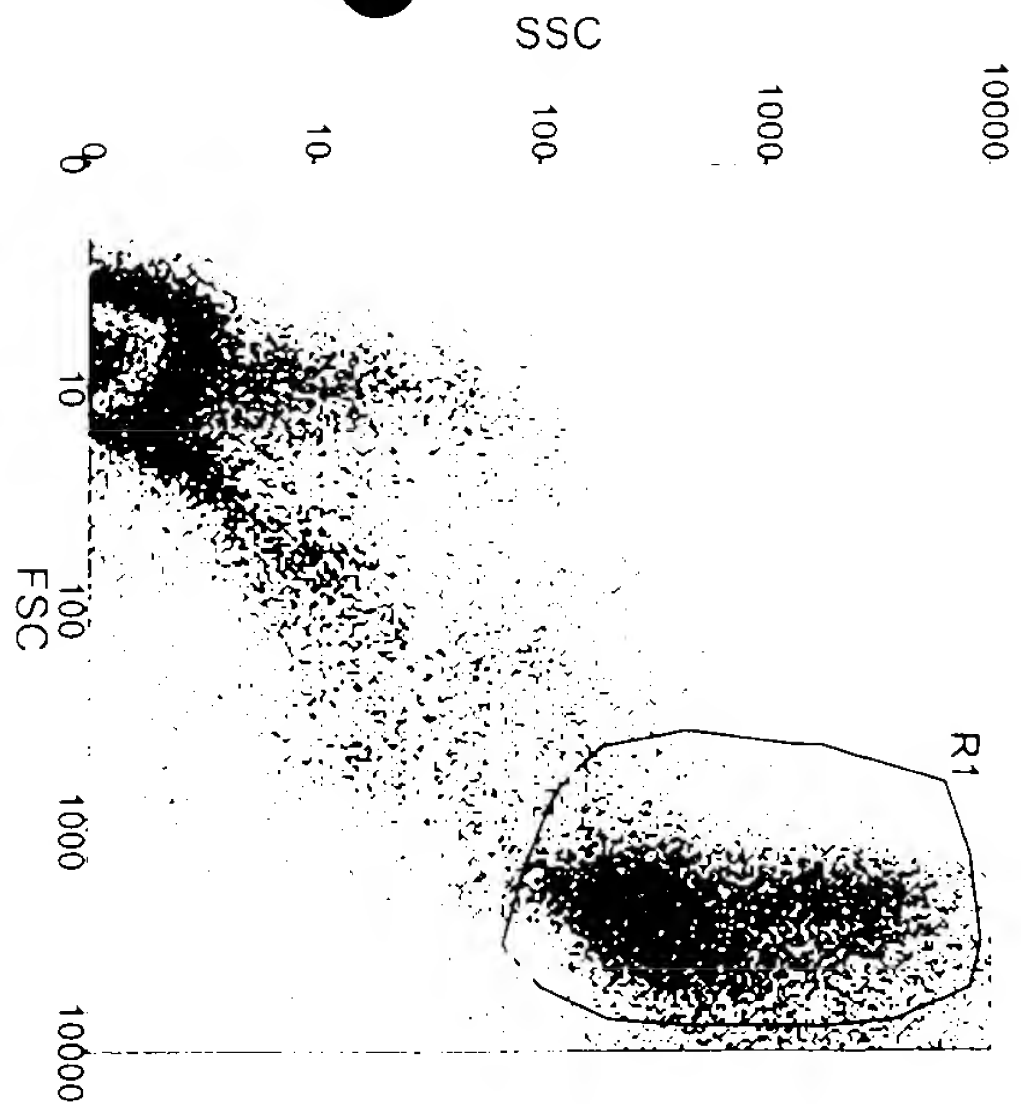
Region	Count	%	Mean
Total	8385	100.00	155.64, 32.50
R4	6764	80.67	143.52, 32.12



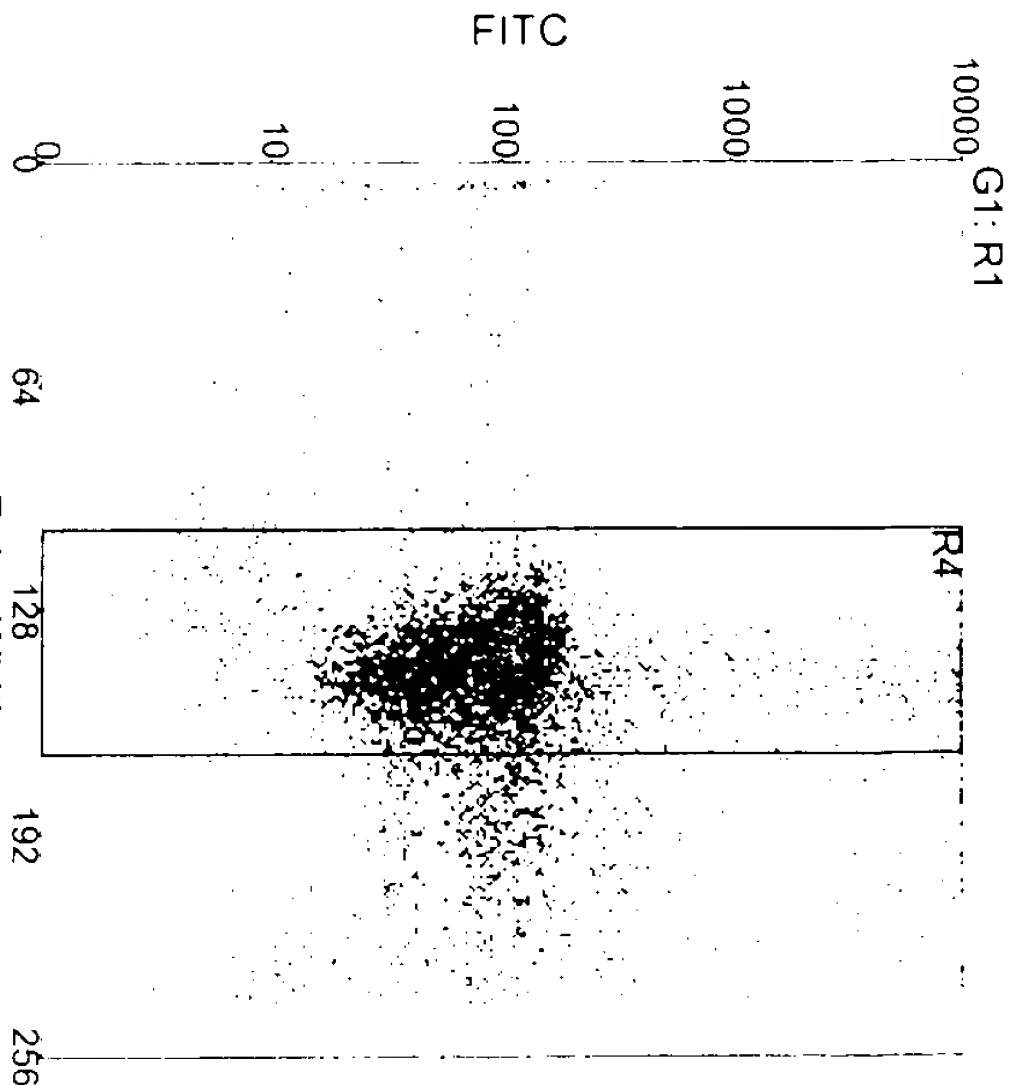
Region	Count	%	Mean
Total	6764	100.00	886.10, 32.12
R2	6753	99.84	886.72, 31.10
R3	11	0.16	507.41, 659.13



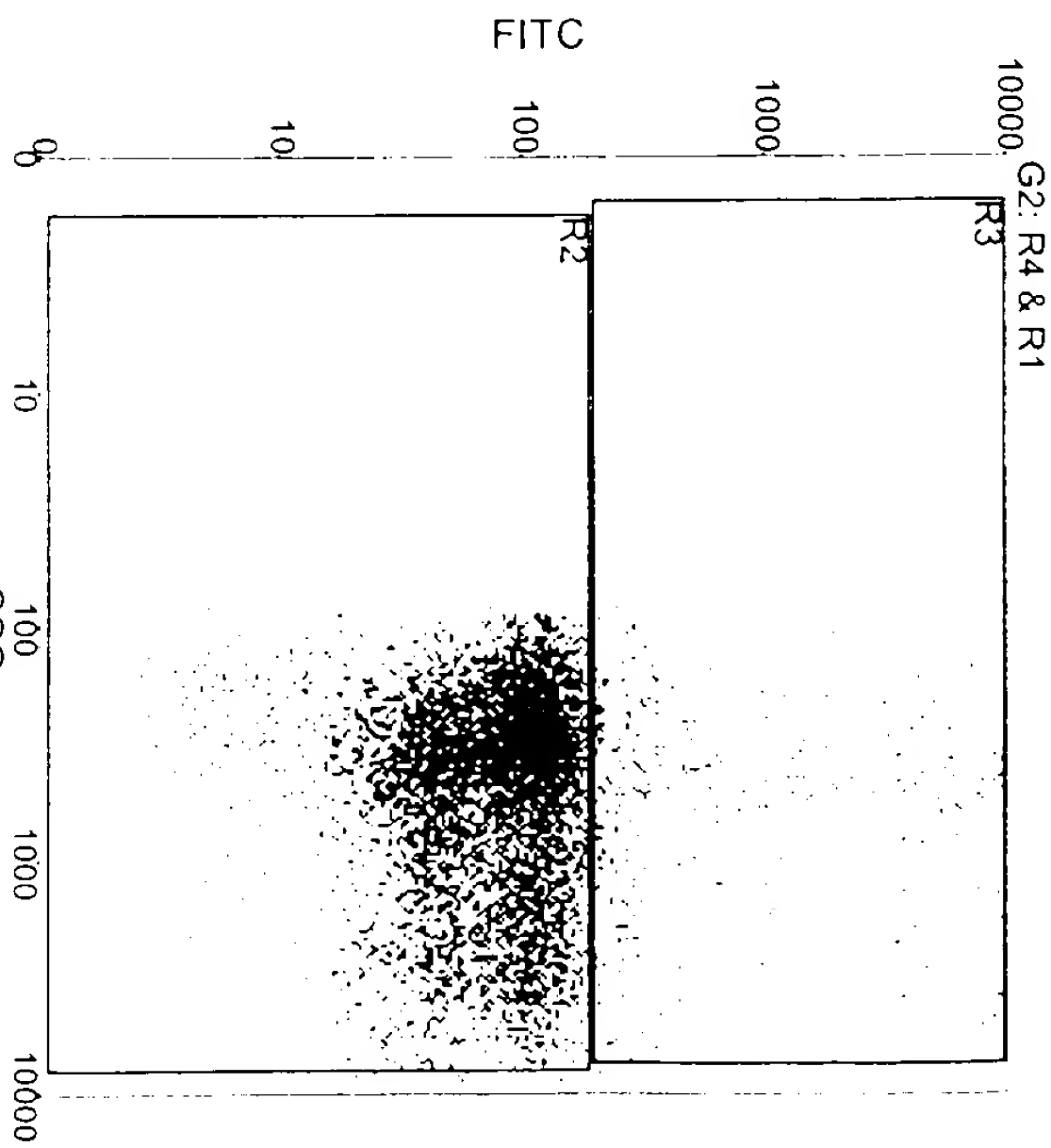
Region	Count	%	Mean
Total	6764	100.00	32.12



Region	Count	%	Mean
Total	50000	100.00	619.91, 204.17
R1	8714	17.43	2700.21, 895.86

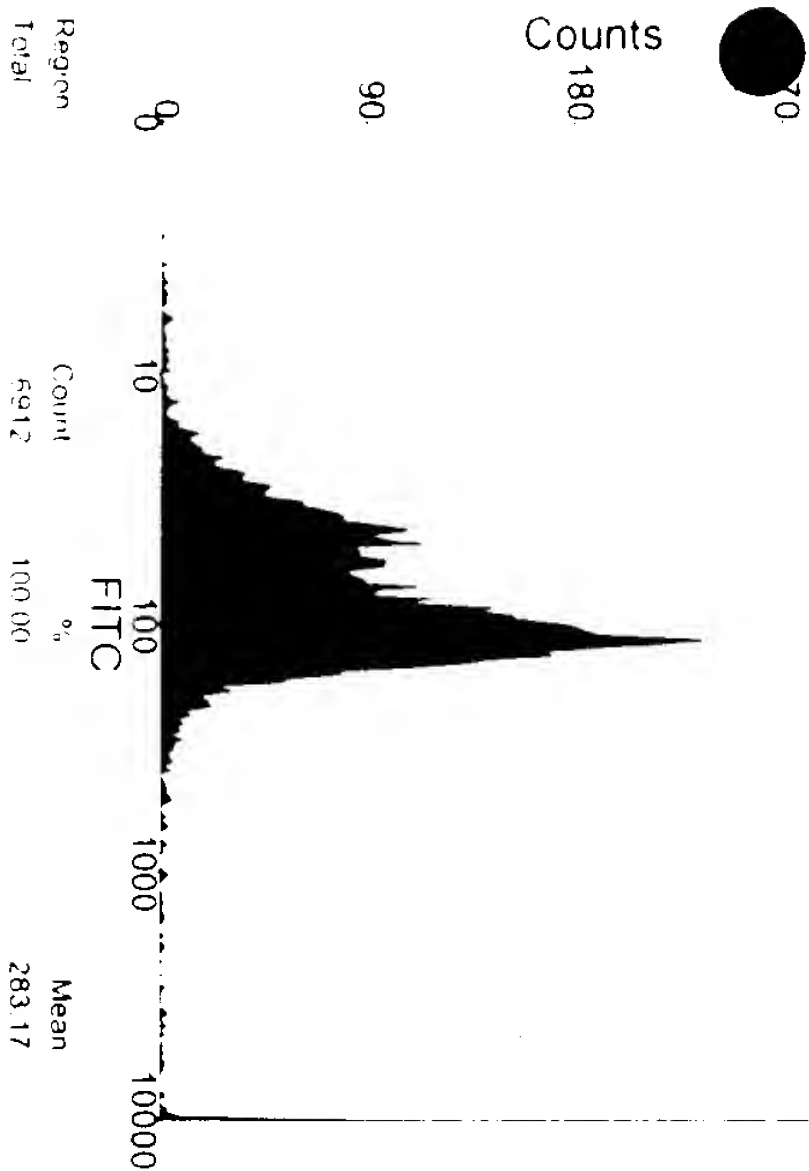


Region	Count	%	Mean
Total	8744	100.00	156.37, 300.47
R4	6912	79.05	142.58, 283.17

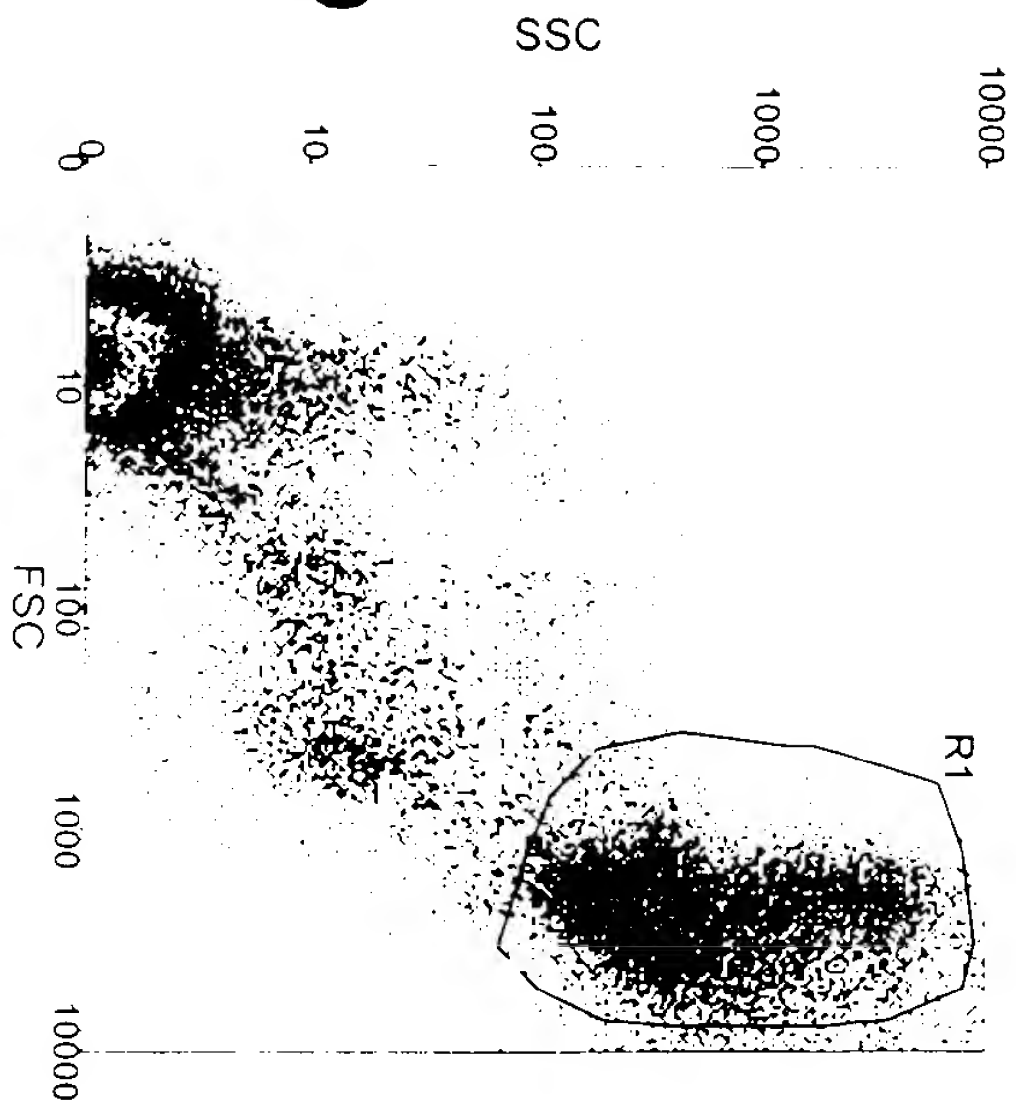


Region	Count	%	Mean
Total	6912	100.00	875.93, 283.17
R2	6464	93.52	863.97, 83.17
R3	349	5.05	1063.07, 1468.16

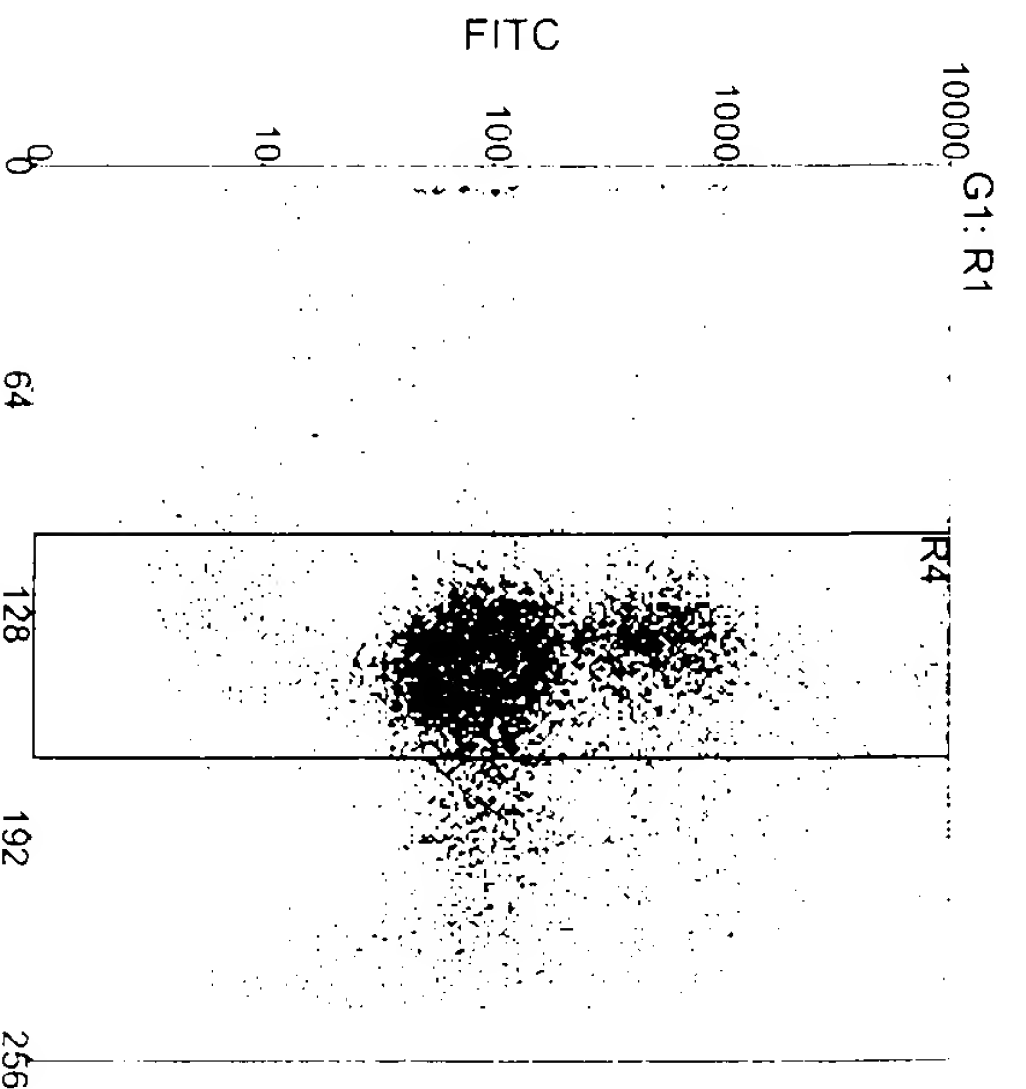
361 G2: R4 & R1



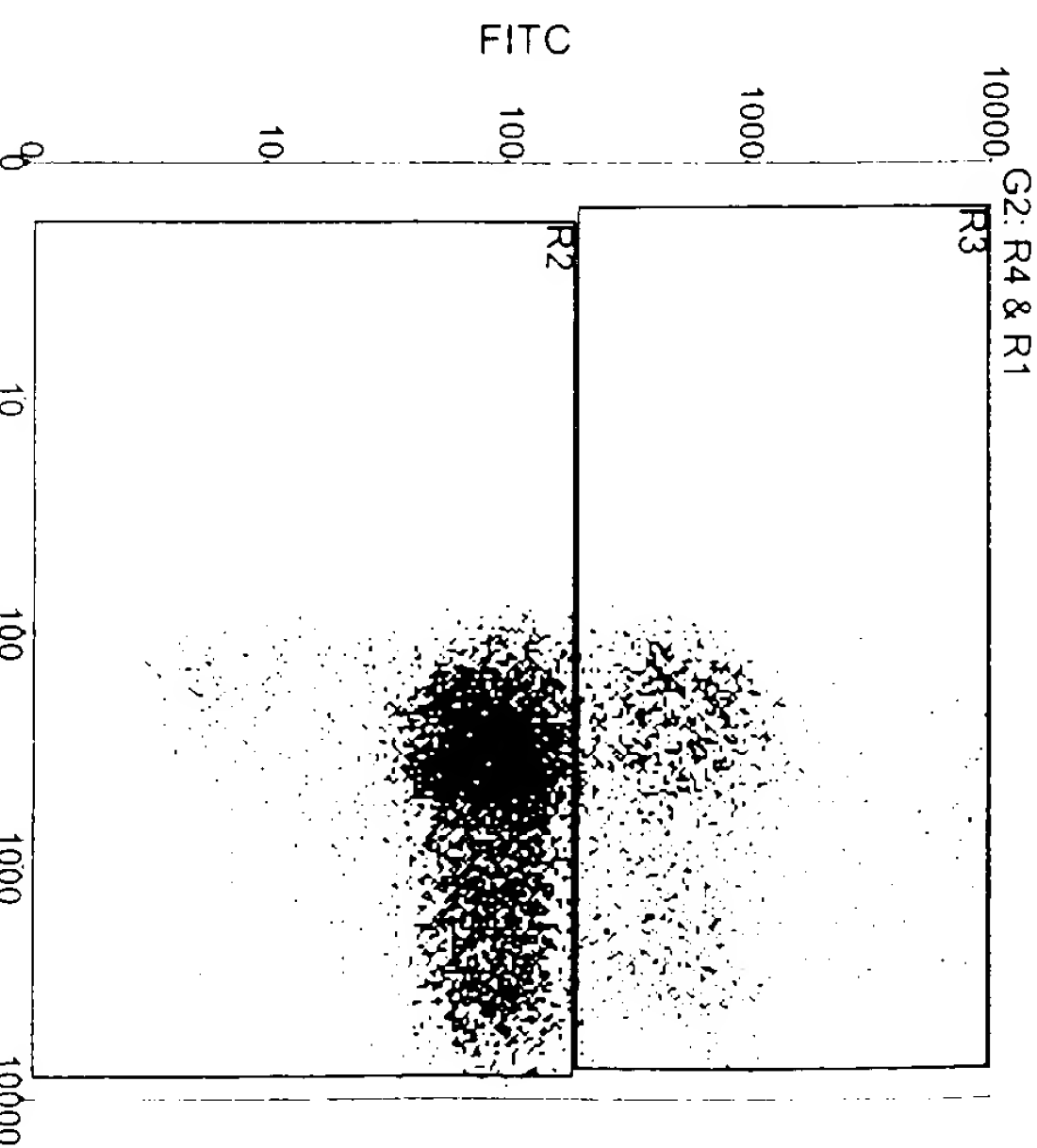
Region	Count	%	Mean
Total	6912	100.00	283.17



Region	Count	%	Mean
Total	50000	100.00	653.81, 221.67
R1	10891	21.78	2486.54, 886.74

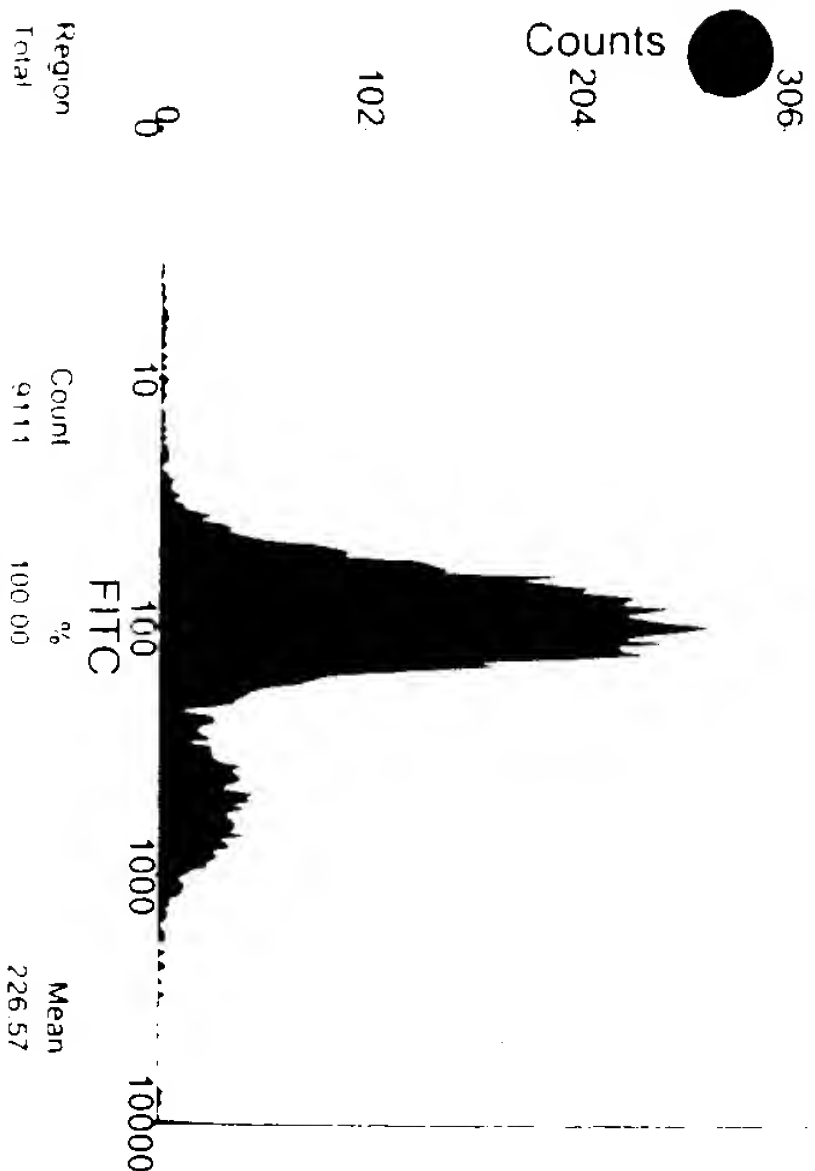


Region	Count	%	Mean
Total	10920	100.00	151.42, 247.95
R4	9111	83.43	140.98, 226.57

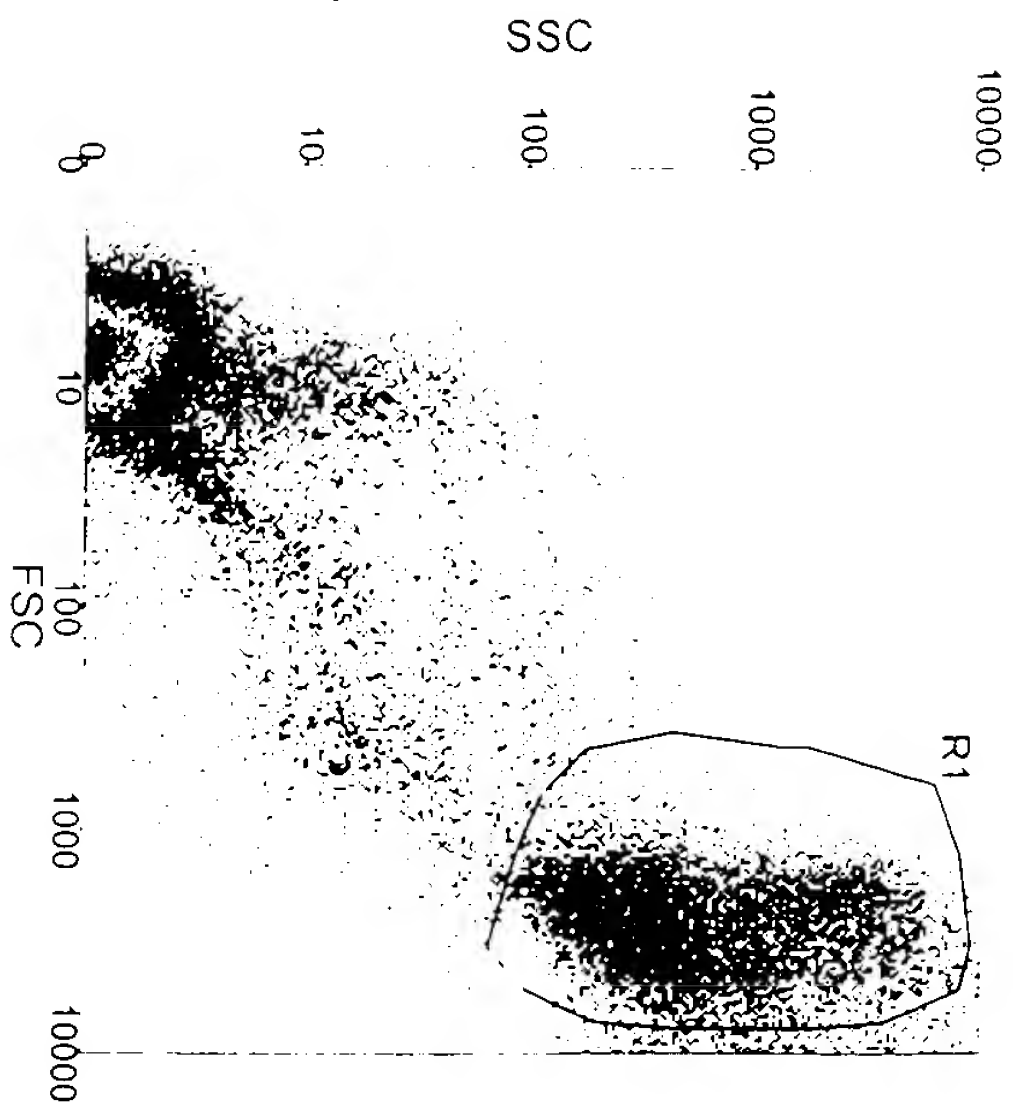


Region	Count	%	Mean
Total	9111	100.00	869.49, 226.57
R2	7448	81.75	876.97, 90.06
R3	1627	17.86	837.18, 648.93

409 G2: R4 & R1

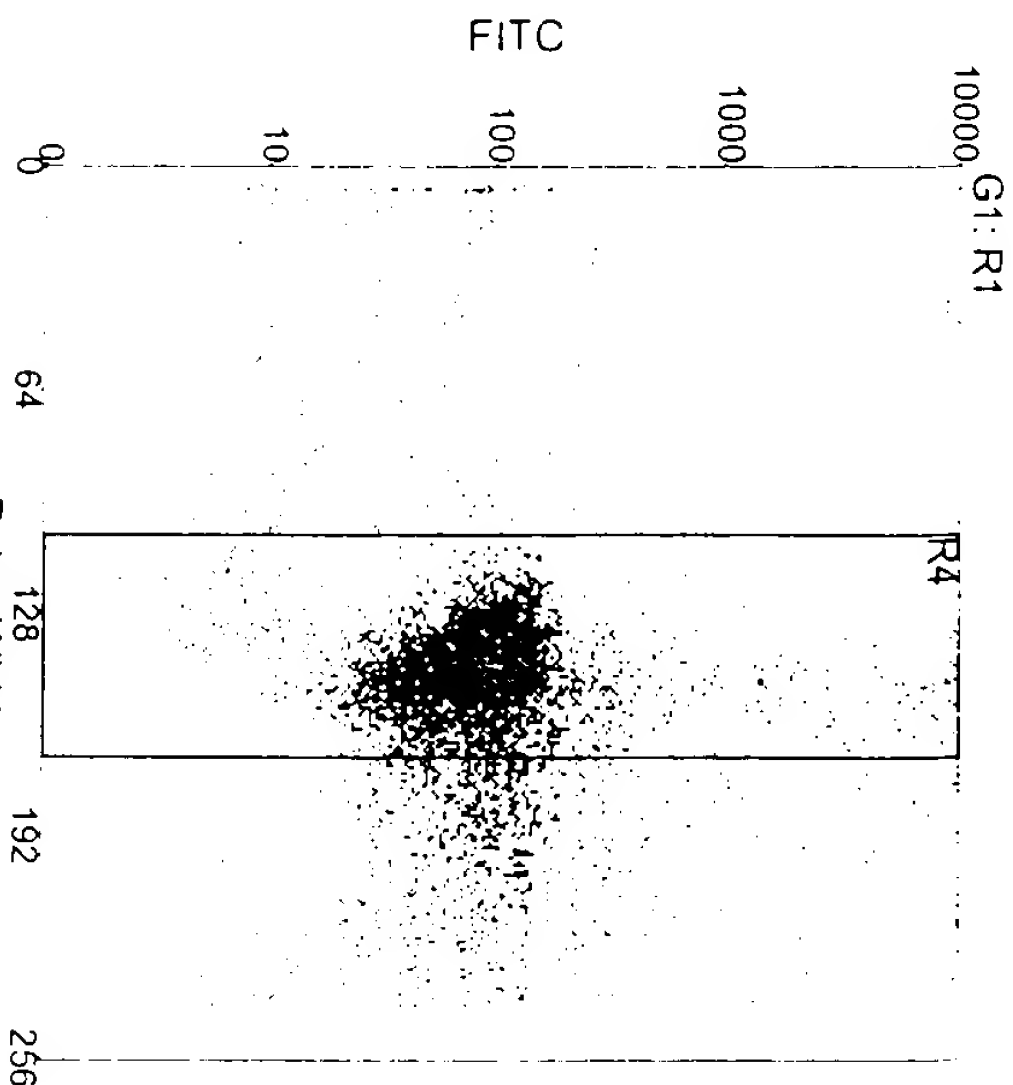


Region	Count	%	Mean
Total	9111	100.00	226.57

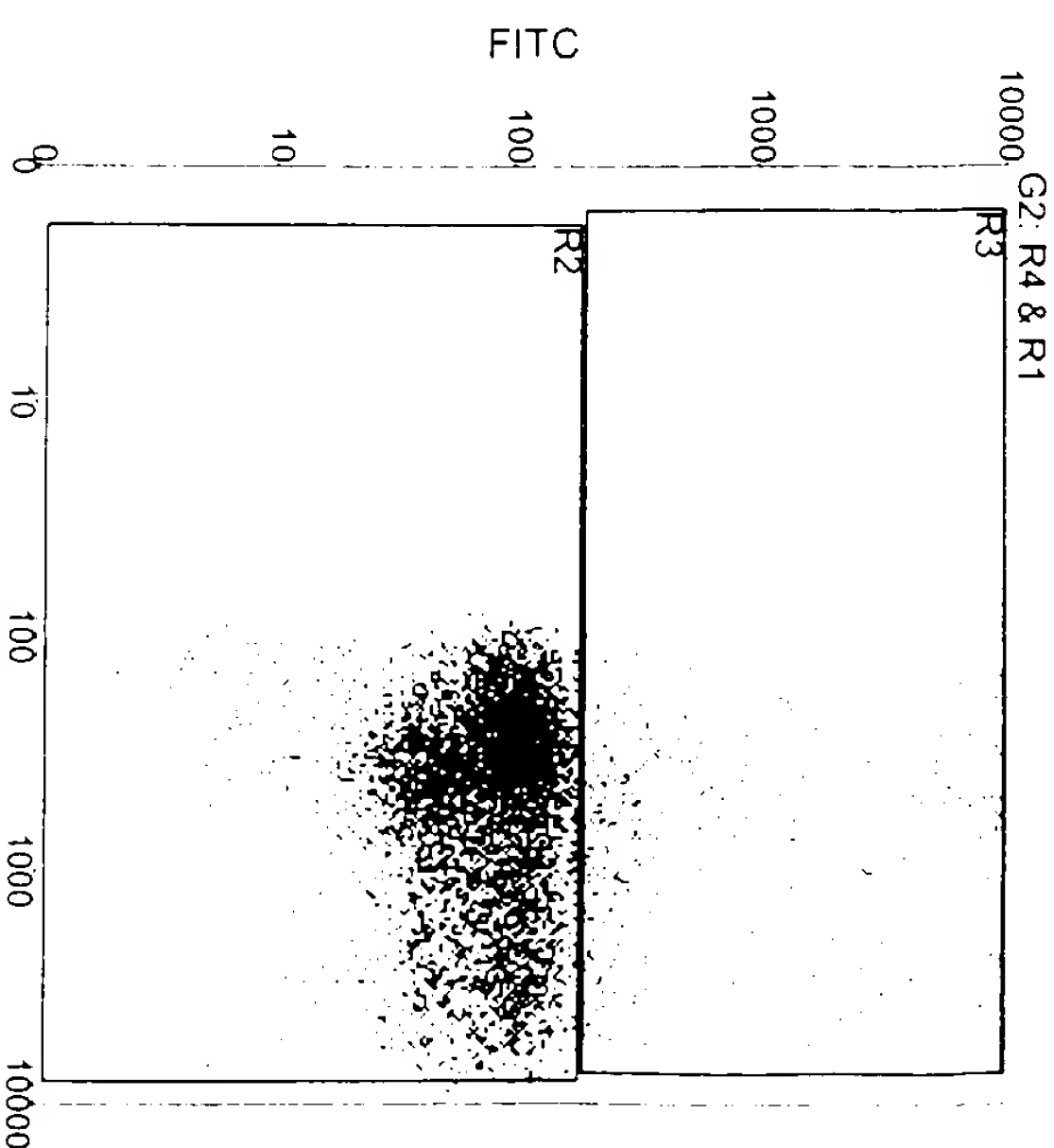


Region	Count	%	Mean
Total	50000	100.00	619.08, 197.16
R1	8650	17.32	2737.25, 902.77

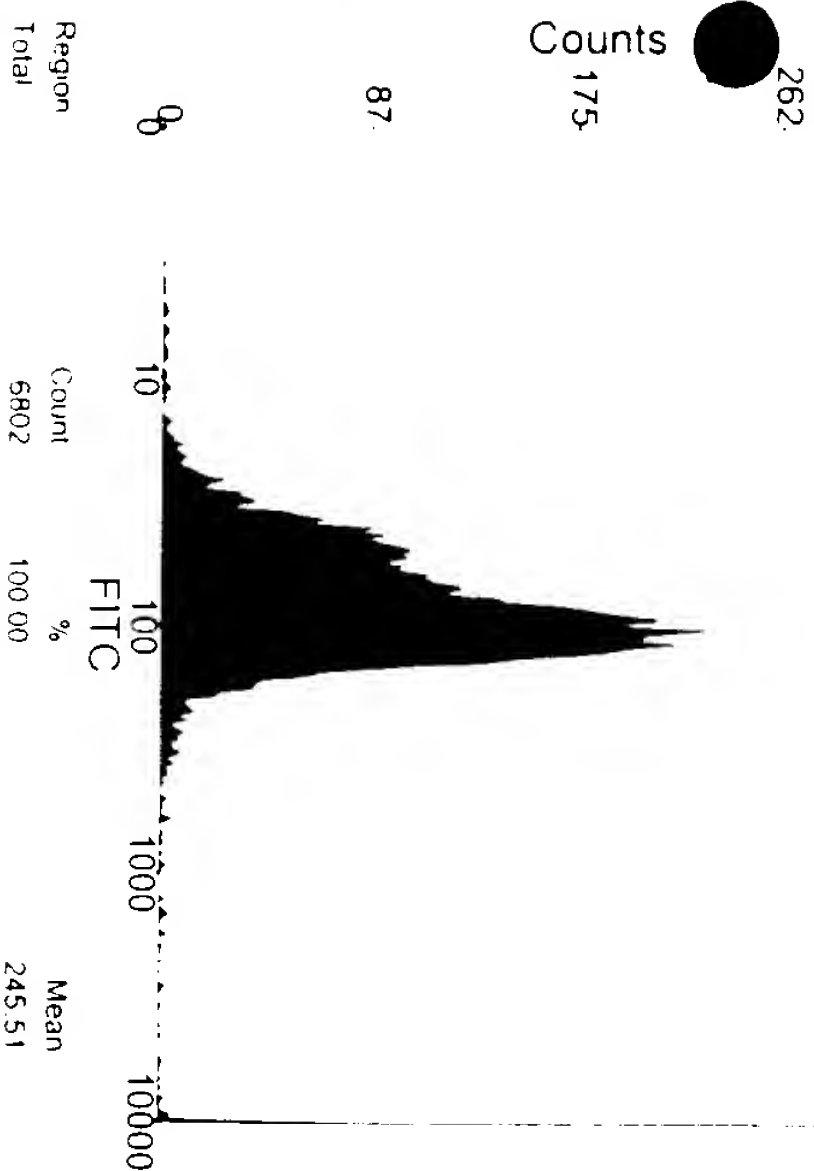
350 G2: R4 & R1



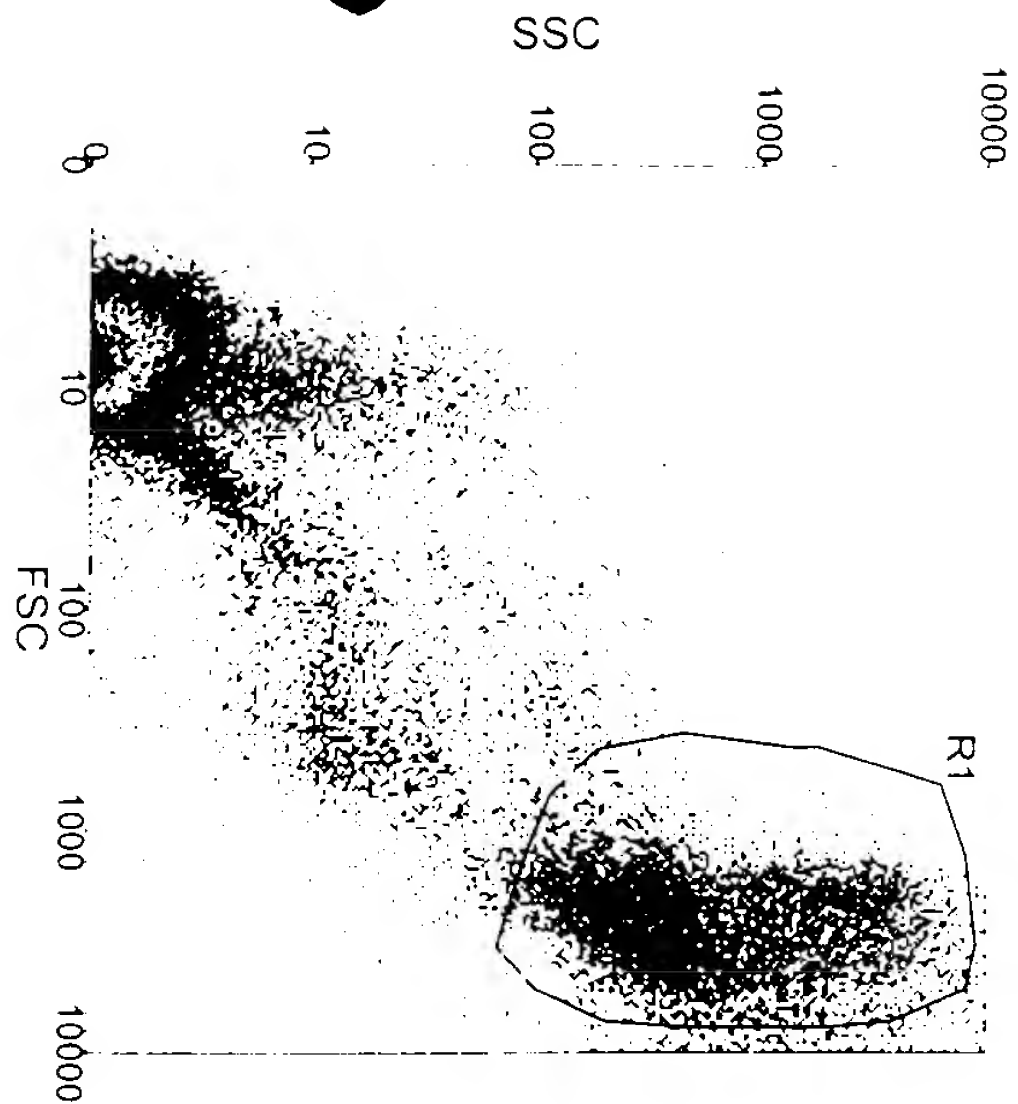
Region	Count	%	Mean
Total	8695	100.00	157.27, 278.34
R4	6802	78.23	142.89, 245.51



Region	Count	%	Mean
Total	6802	100.00	870.77, 245.51
R2	6438	94.65	860.34, 85.49
R3	286	4.20	1055.34, 1317.32

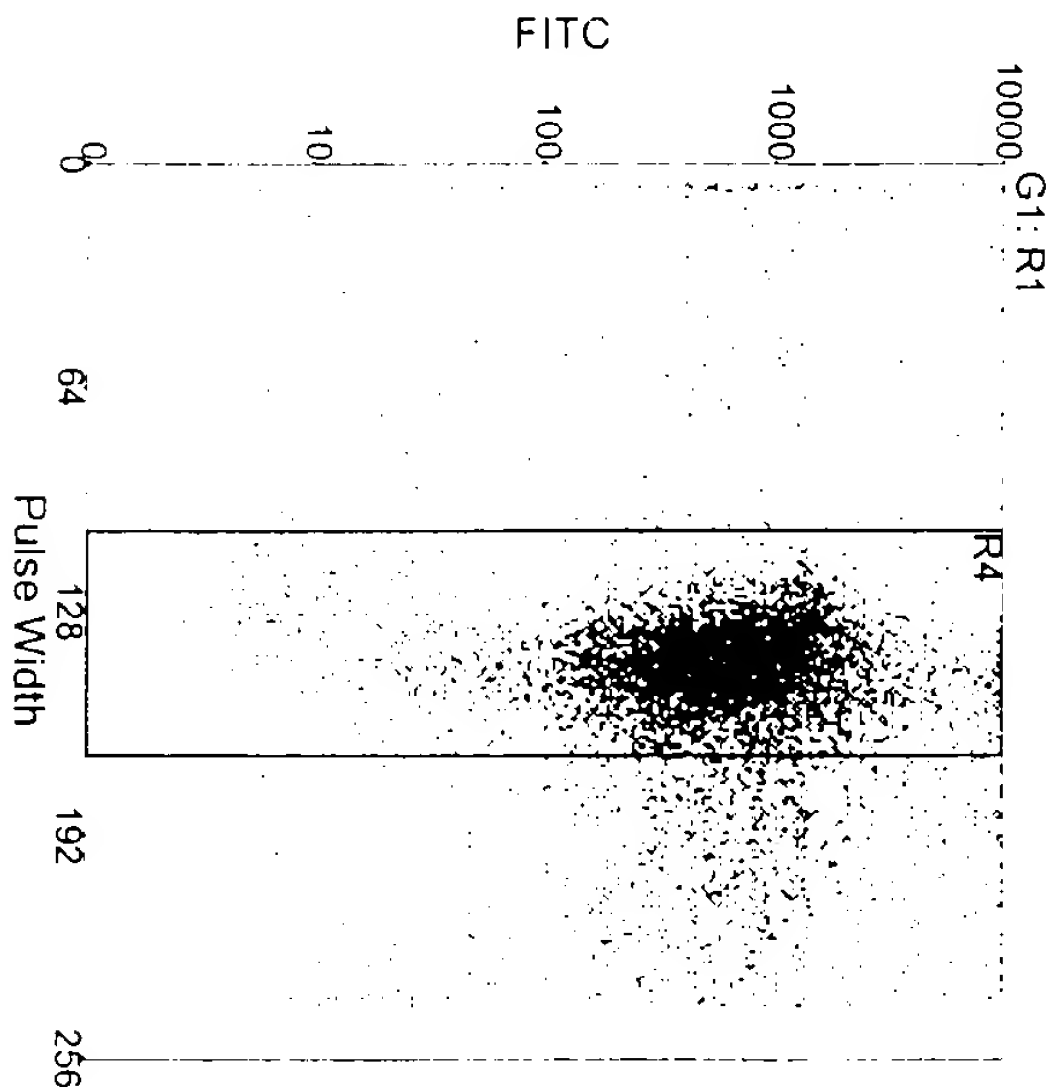


Region	Count	%	Mean
Total	6802	100.00	245.51

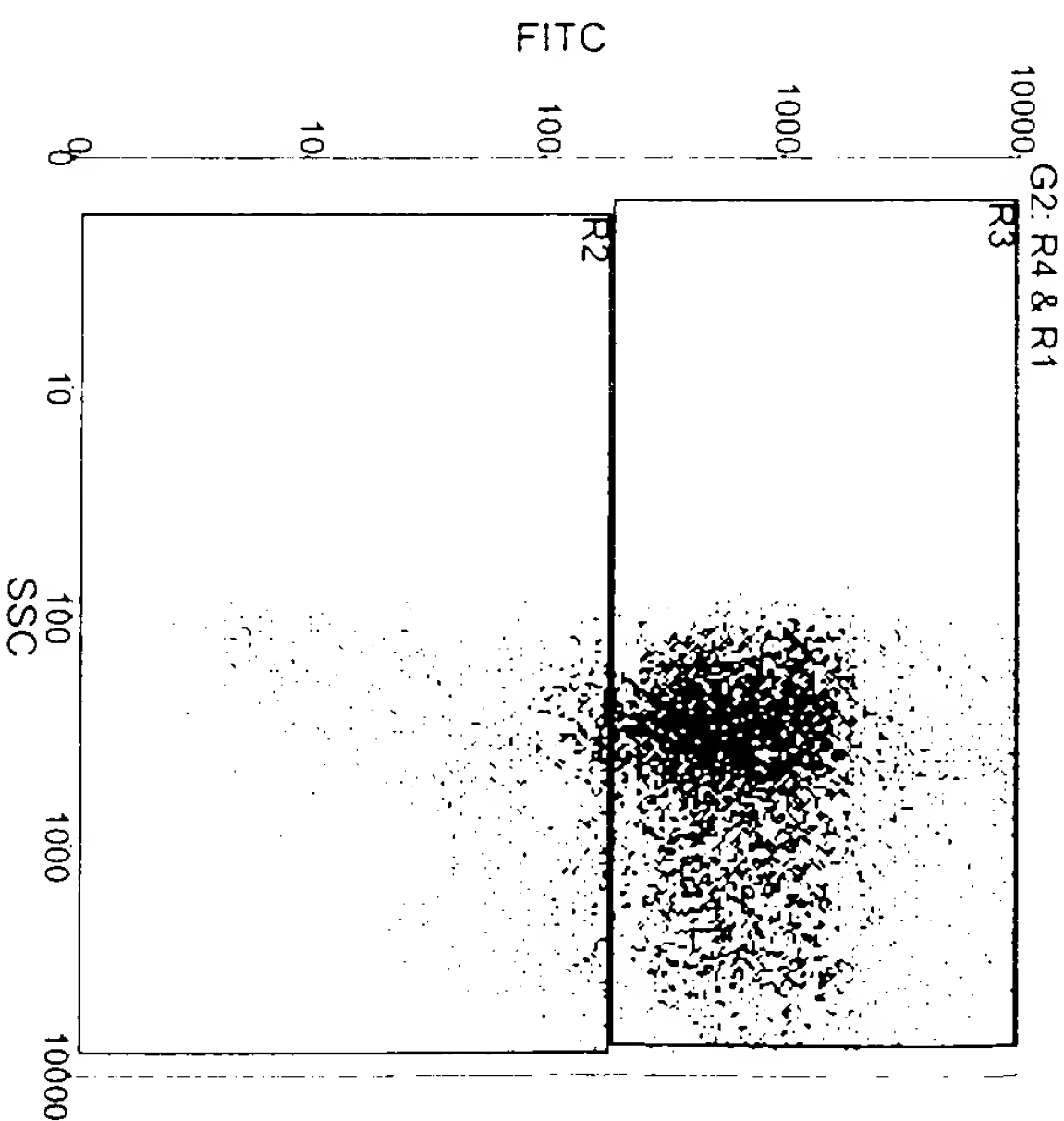


Region	Count	%	Mean
Total	50000	100.00	516.58, 135.81
R1	8965	17.93	2723.48, 879.04

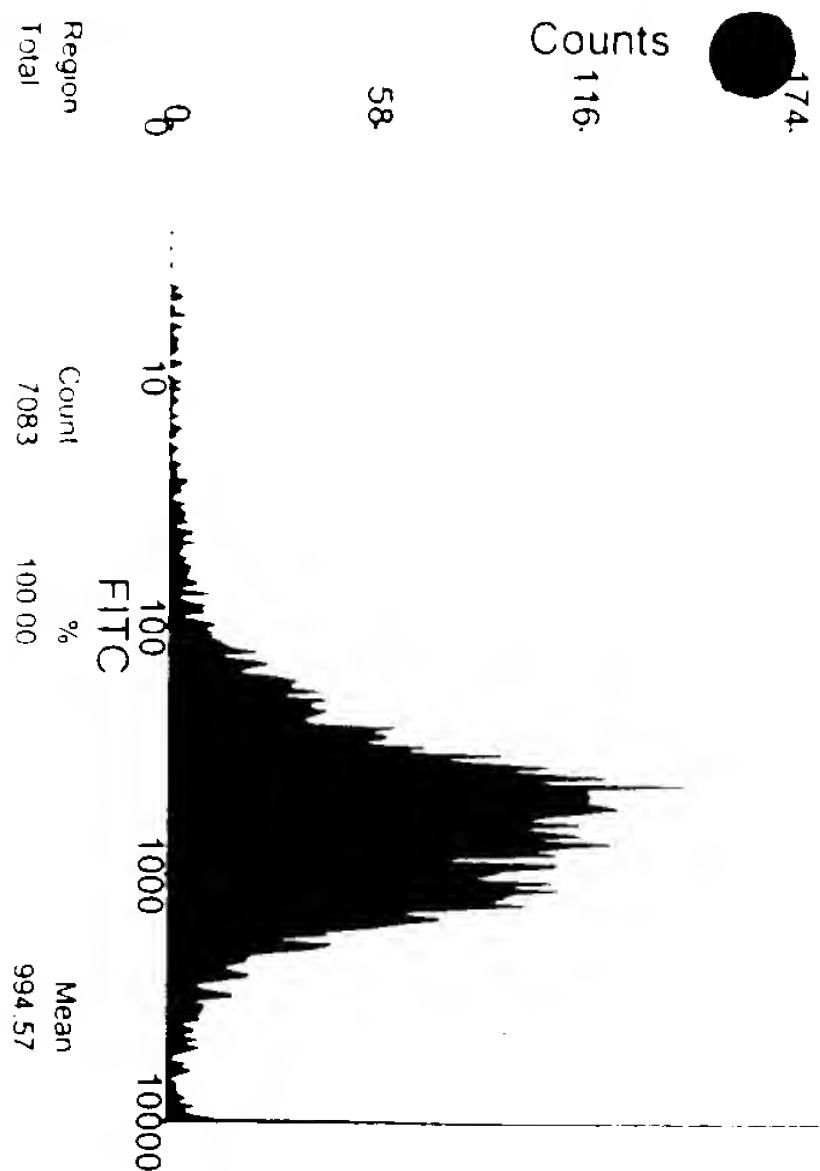
232. G2: R4 & R1



Region	Count	%	Mean
Total	8994	100.00	156.57, 1082.71
R4	7083	78.75	142.66, 994.57



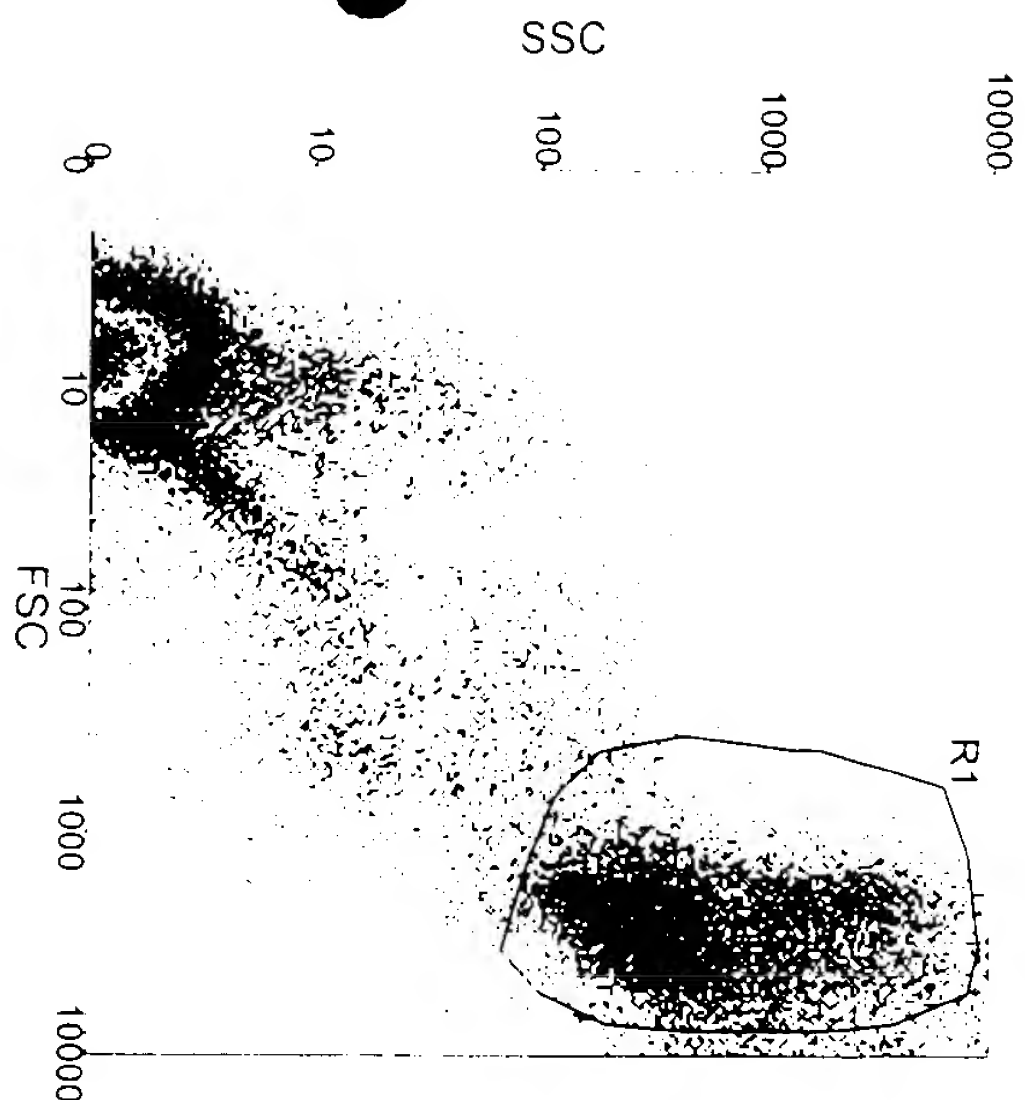
Region	Count	%	Mean
Total	7083	100.00	845.48, 994.57
R2	759	10.72	764.37, 104.30
R3	6211	87.69	852.70, 950.42



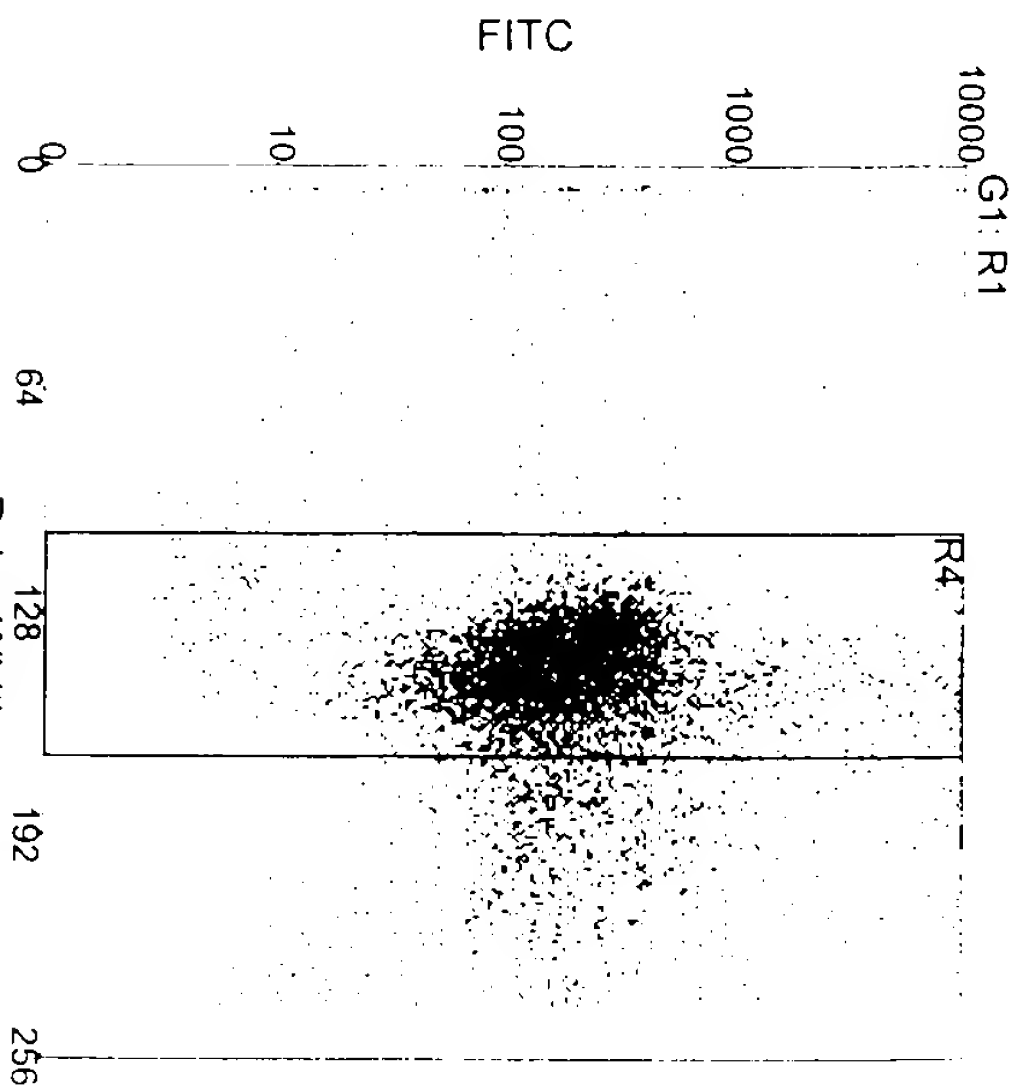
Counts 116.

58.

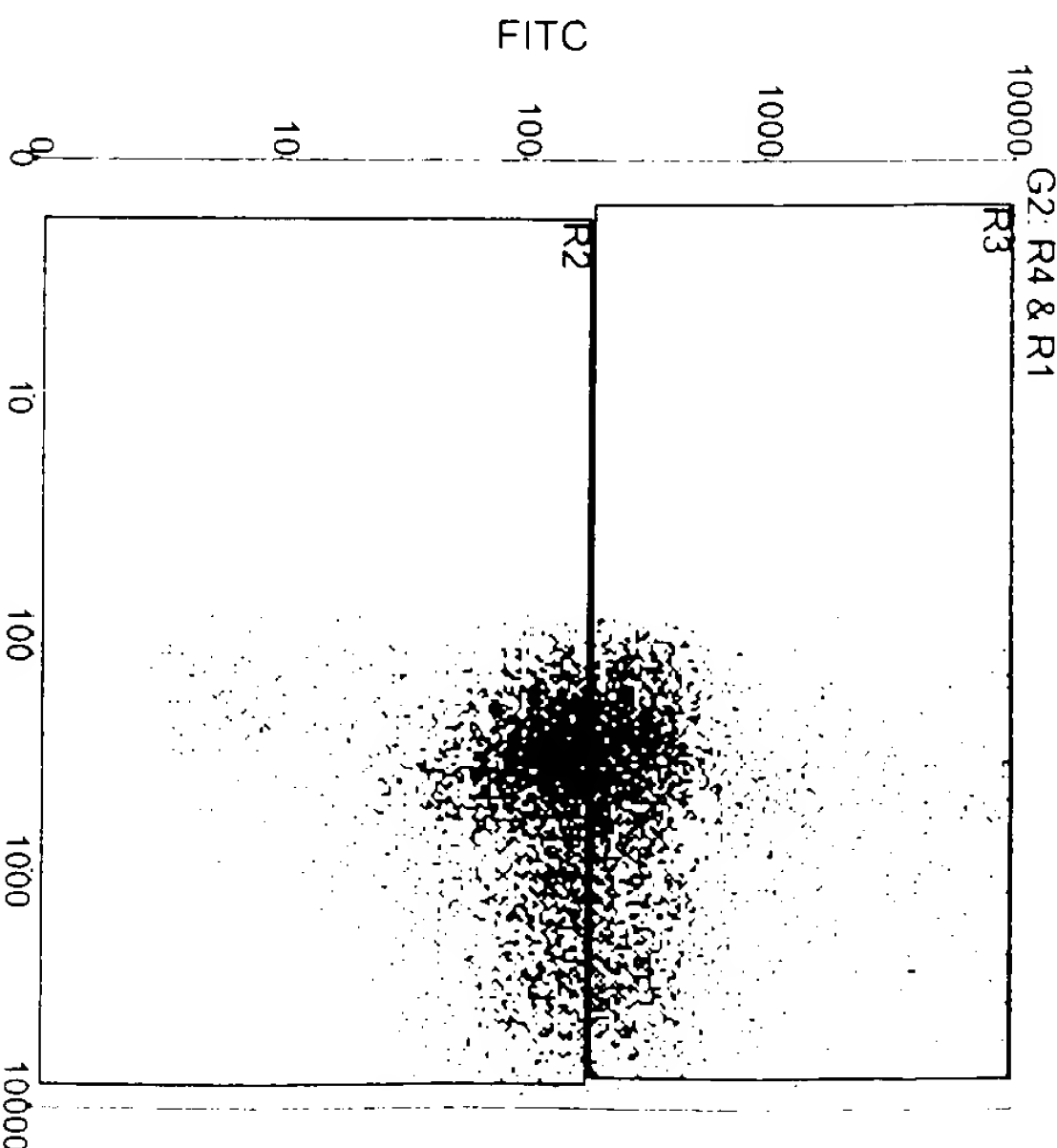
Region	Count	%	Mean
Total	7083	100.00	994.57



Region	Count	%	Mean
Total	50000	100.00	614.11, 202.03
R1	8893	17.79	2725.28, 886.85

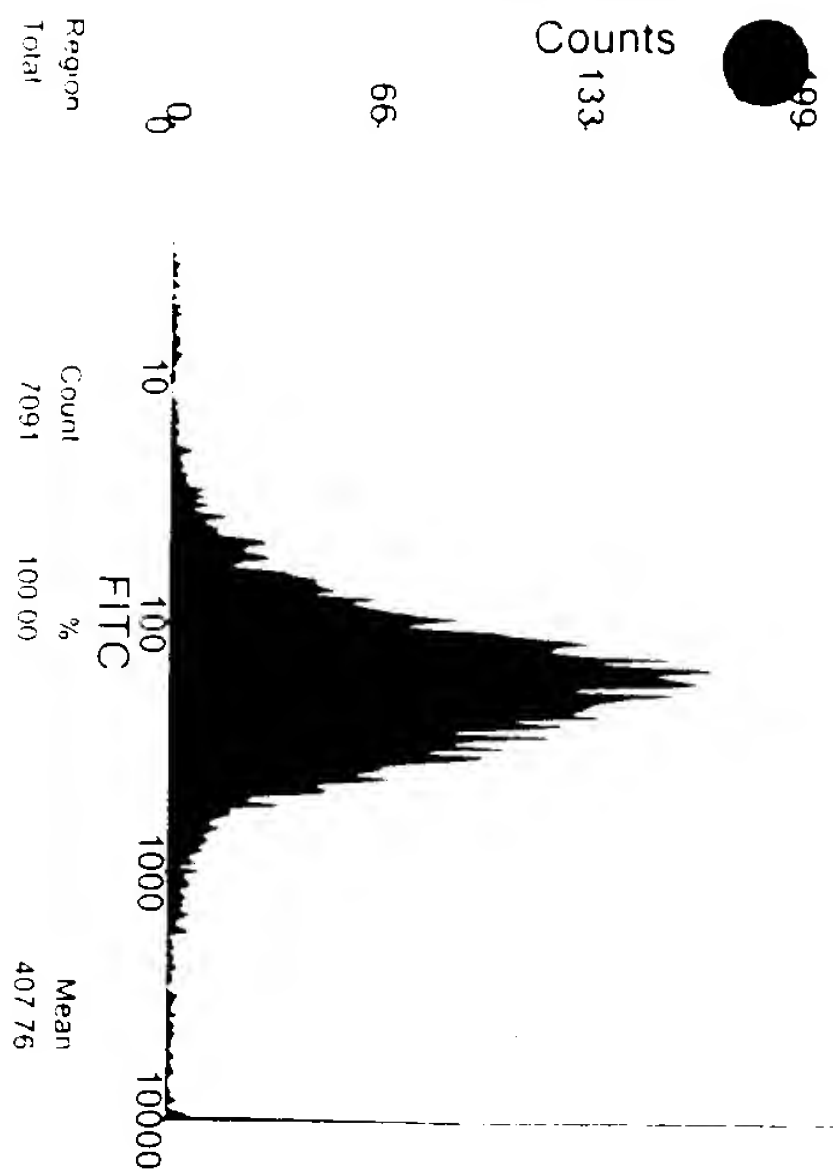


Region	Count	%	Mean
Total	8915	100.00	155.73, 468.50
R4	7091	79.54	142.51, 407.76

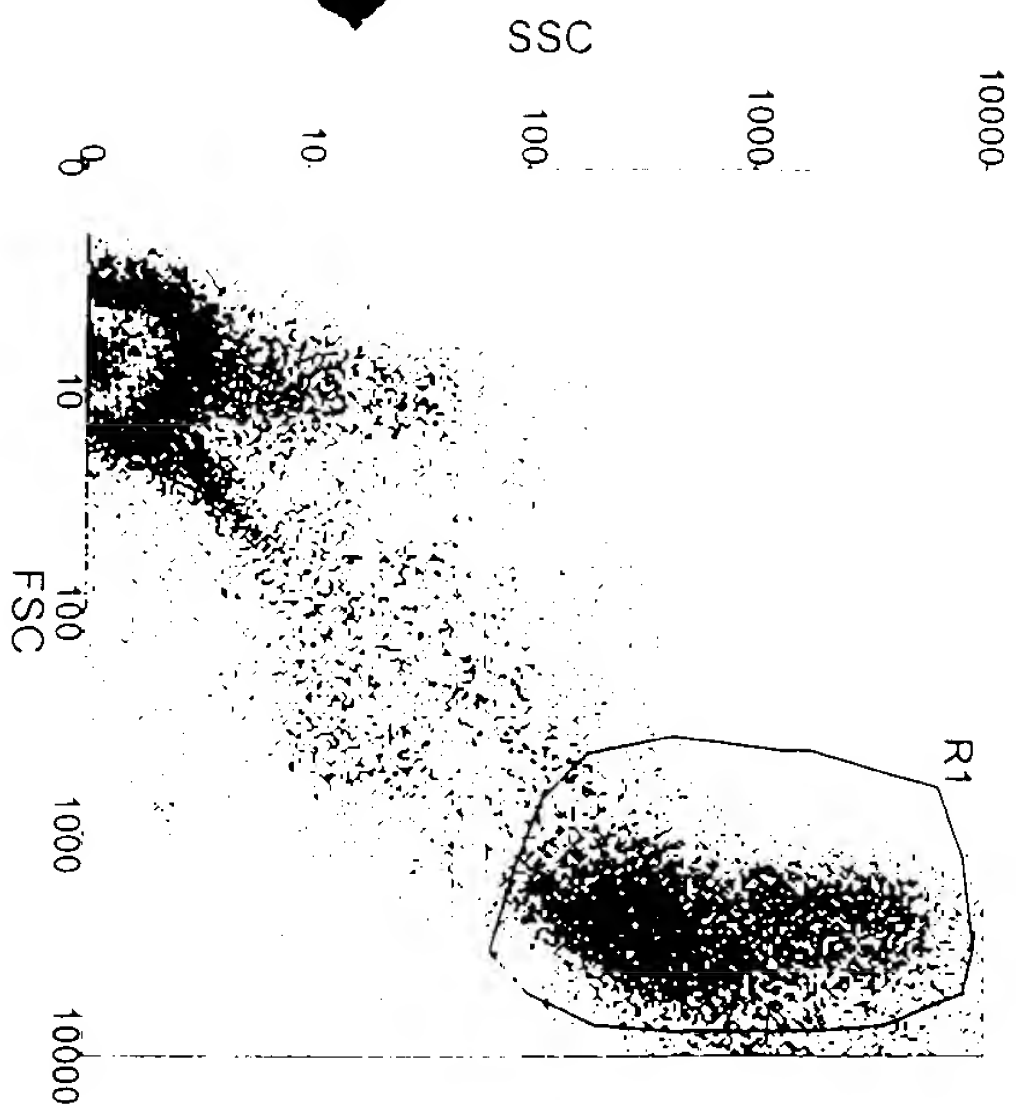


Region	Count	%	Mean
Total	7091	100.00	844.39, 407.76
R2	3930	55.42	809.01, 114.17
R3	3062	43.18	881.24, 497.85

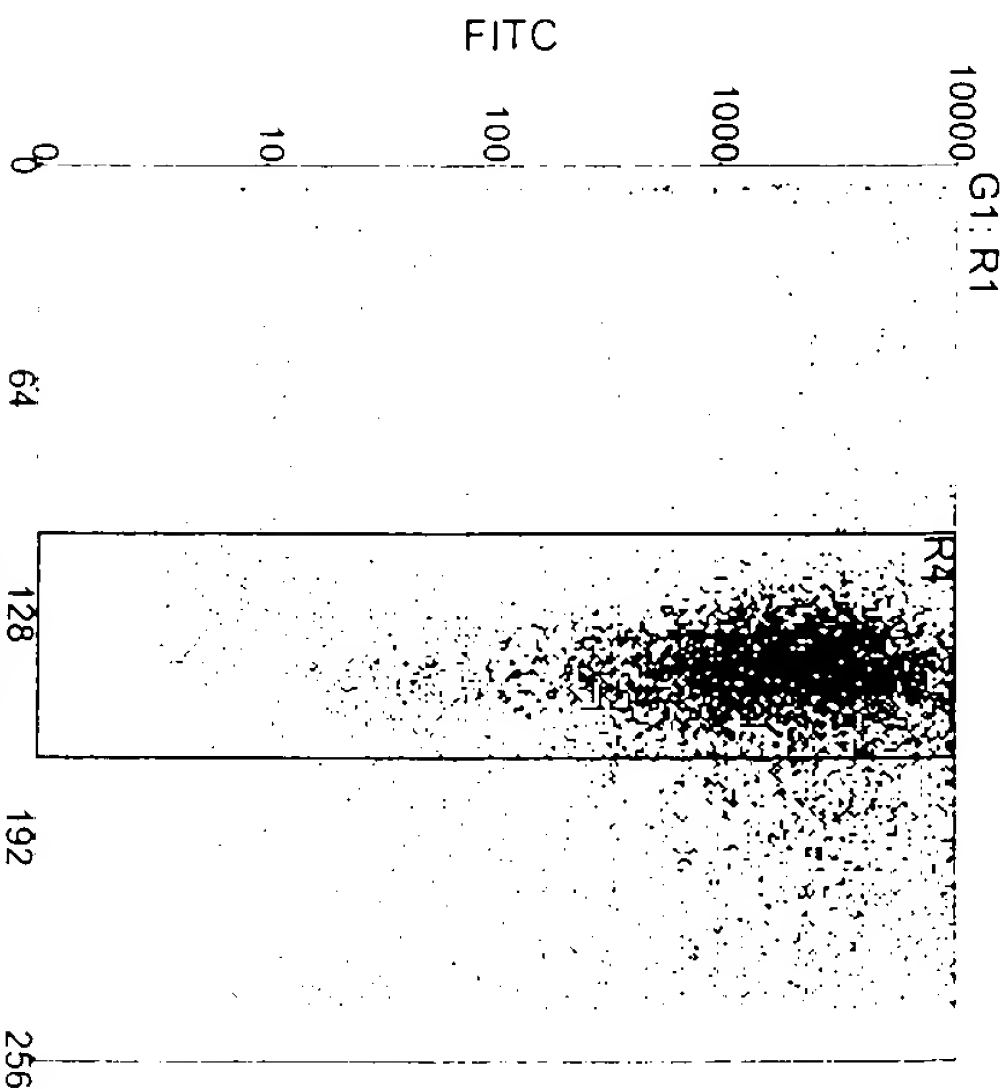
266 G2: R4 & R1



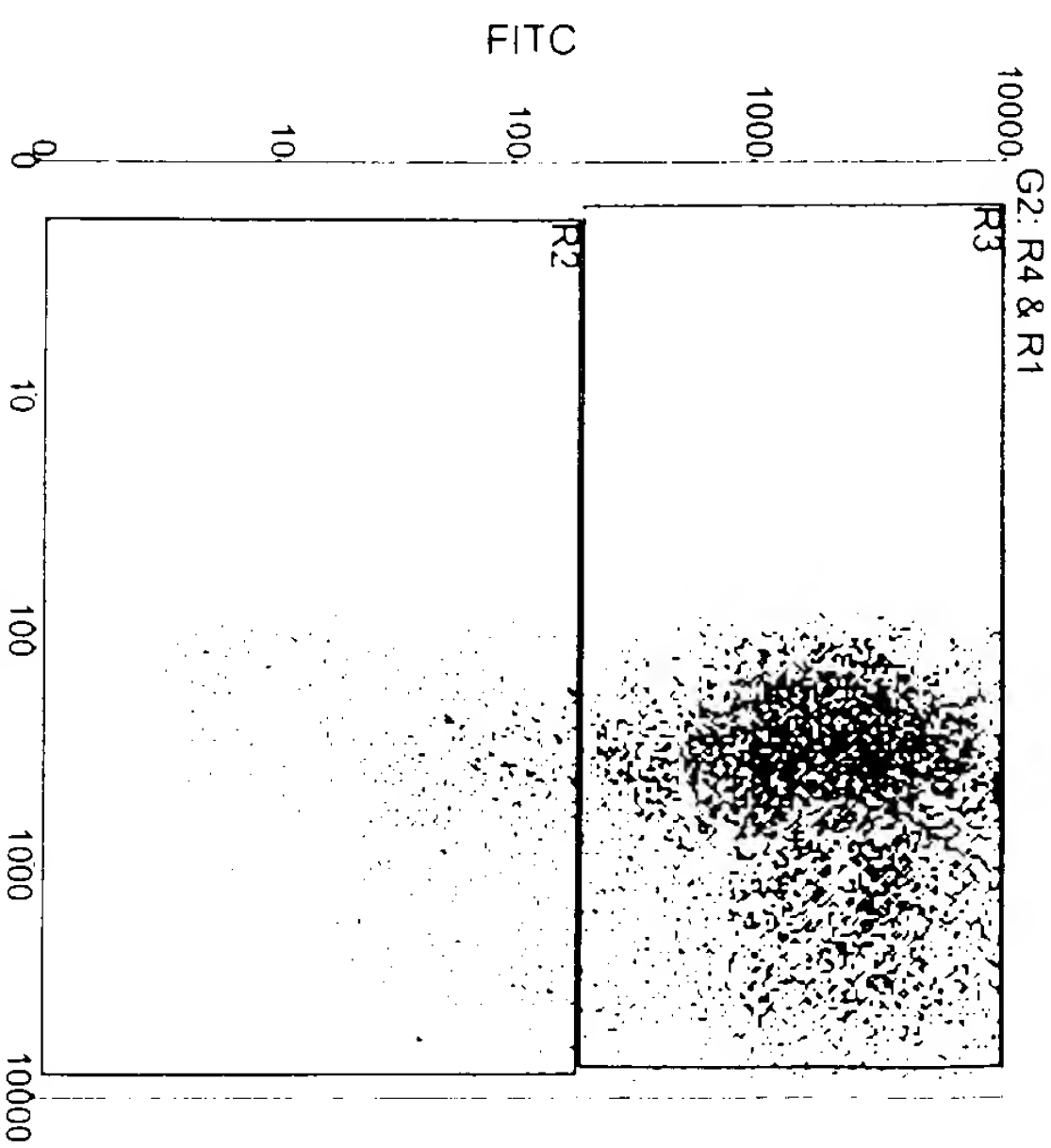
Region	Count	%	Mean
Total	7091	100.00	407.76



Region	Count	%	Mean
Total	50000	100.00	611.90, 201.63
R1	8939	17.88	2737.66, 905.07

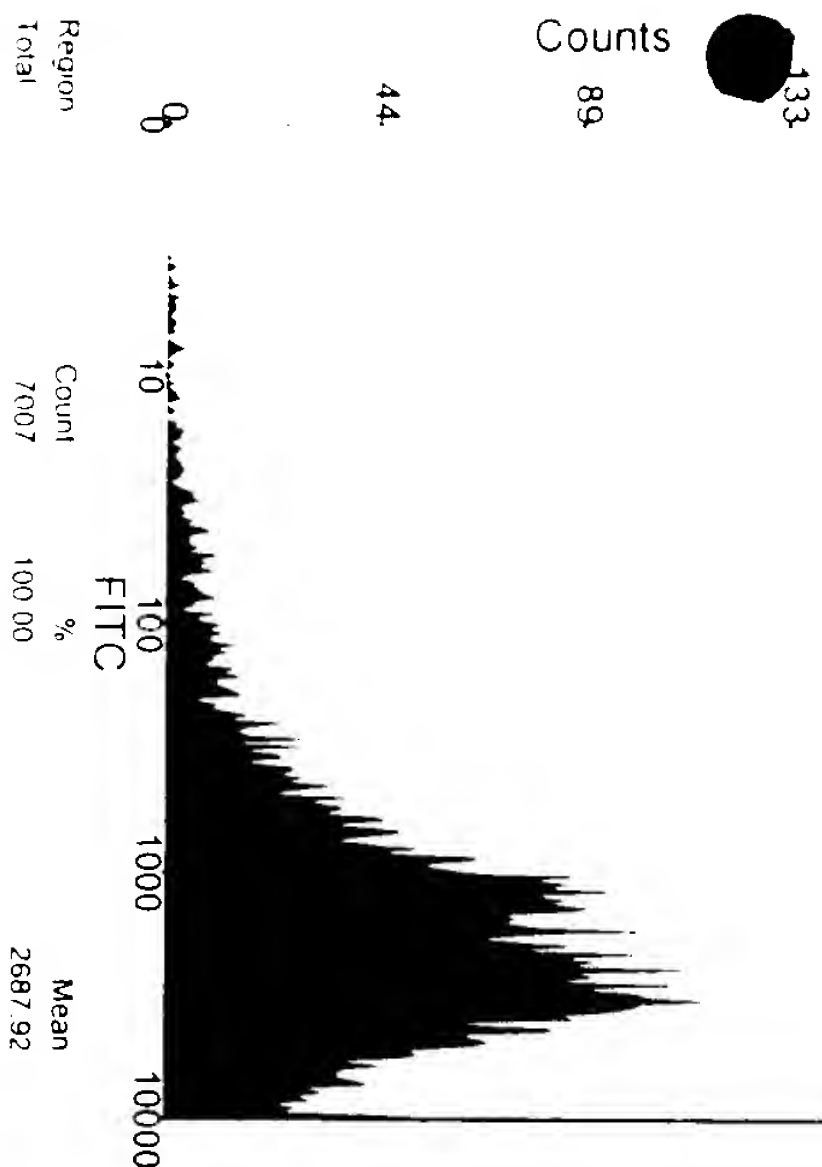


Region	Count	%	Mean
Total	8970	100.00	157.07, 2822.53
R4	7007	78.12	142.94, 2687.92

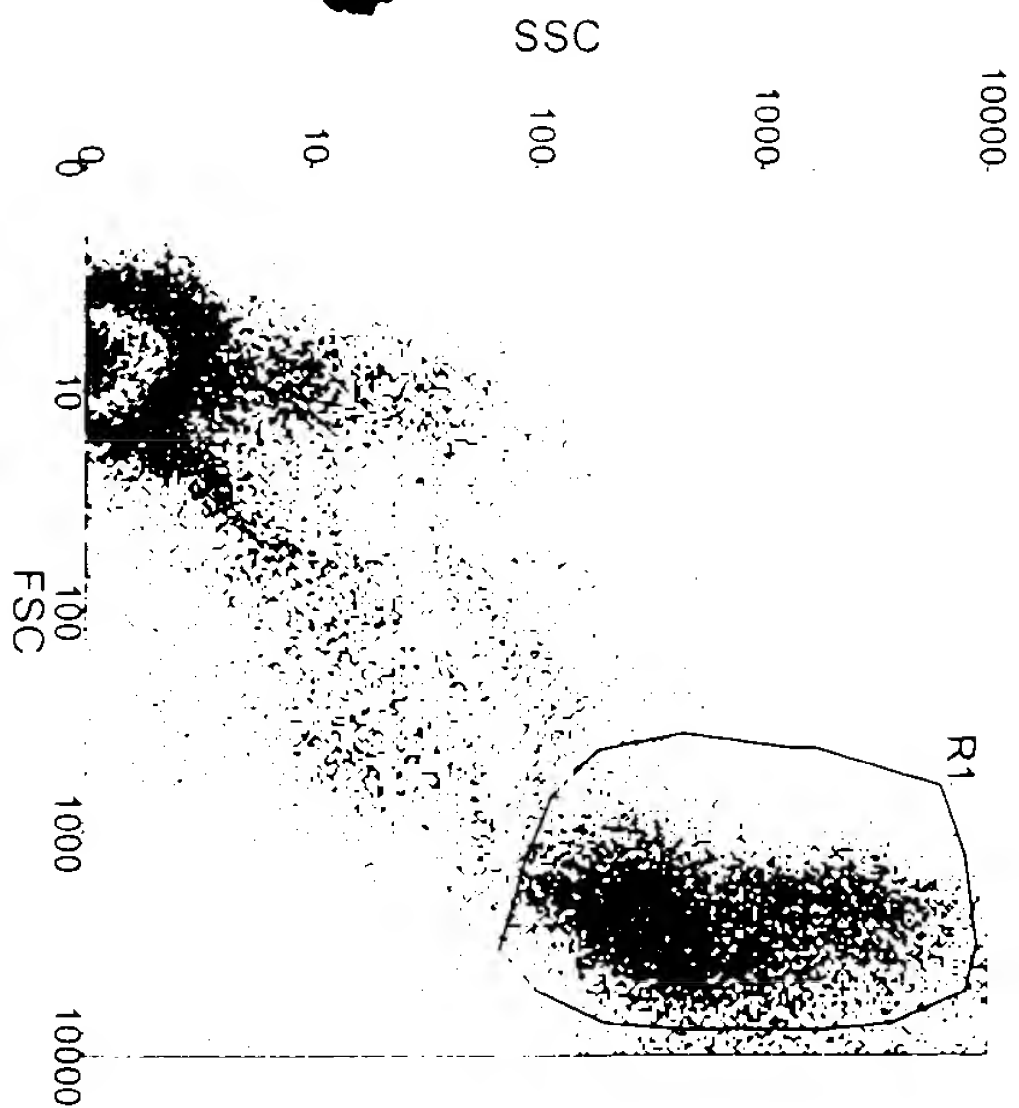


Region	Count	%	Mean
Total	7007	100.00	879.12, 2687.92
R2	529	7.55	824.18, 82.87
R3	6083	86.81	881.79, 2474.10

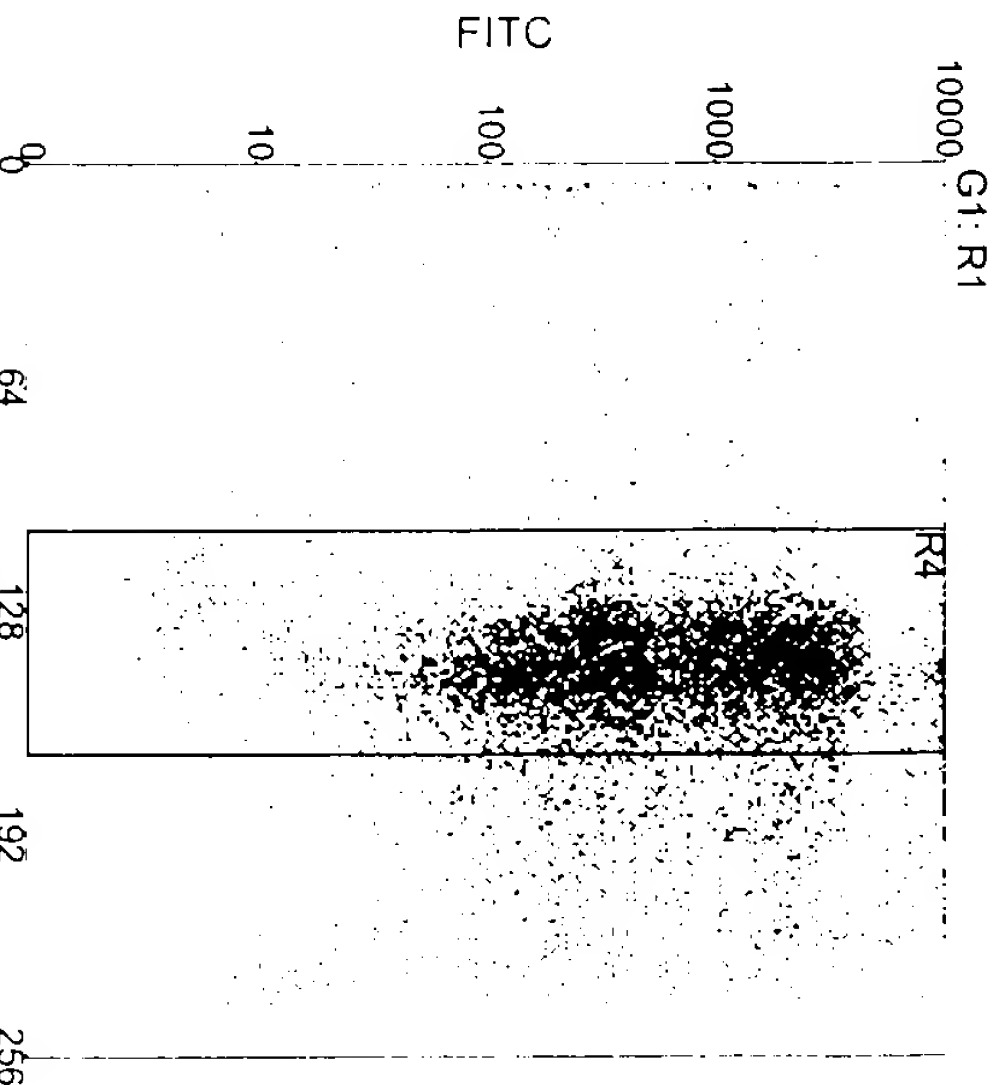
178 G2: R4 & R1



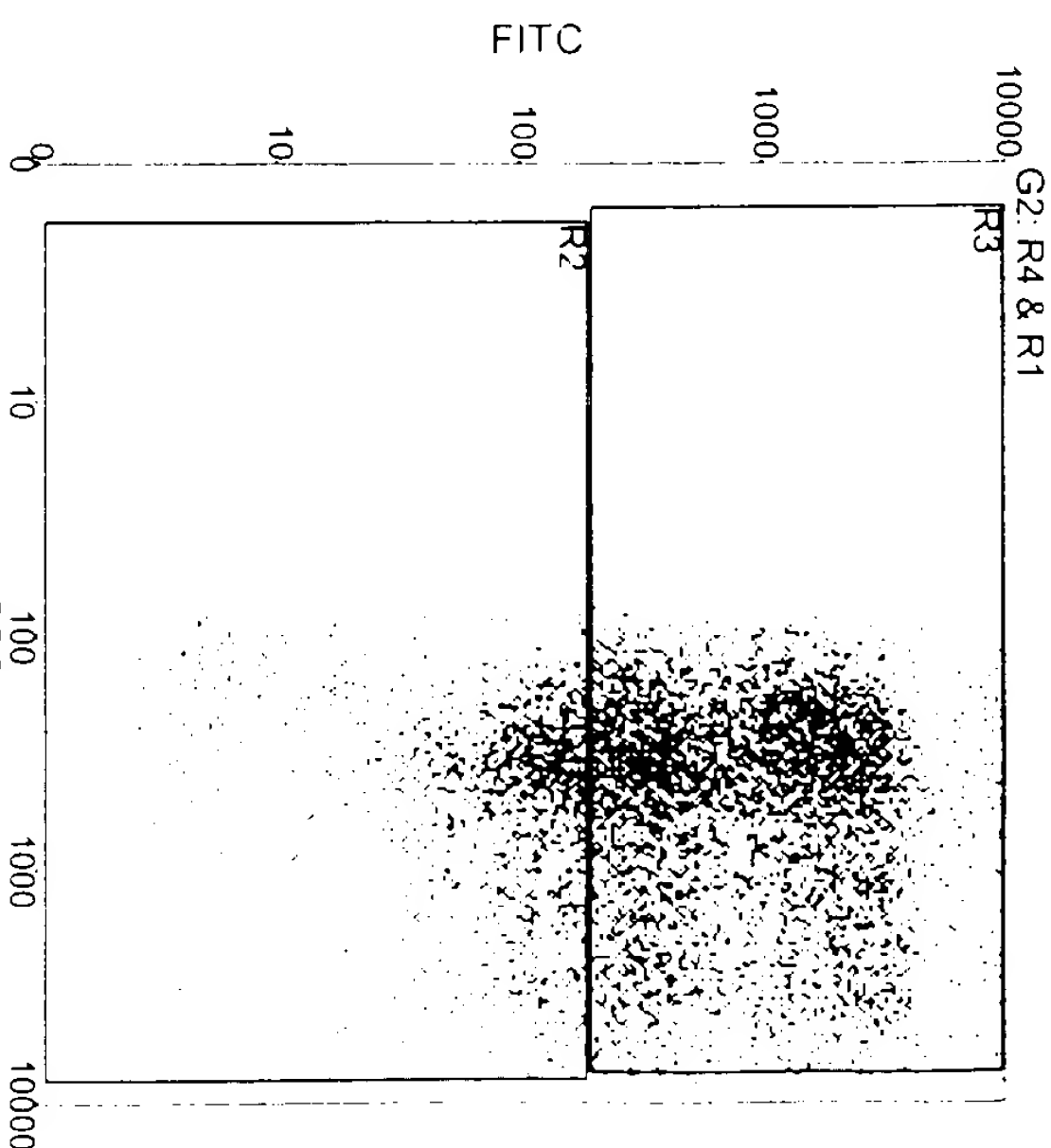
Region	Count	%	Mean
Total	7007	100.00	2687.92



Region	Count	%	Mean
Total	50000	100.00	609.38, 197.20
R1	9020	18.04	2716.36, 880.21

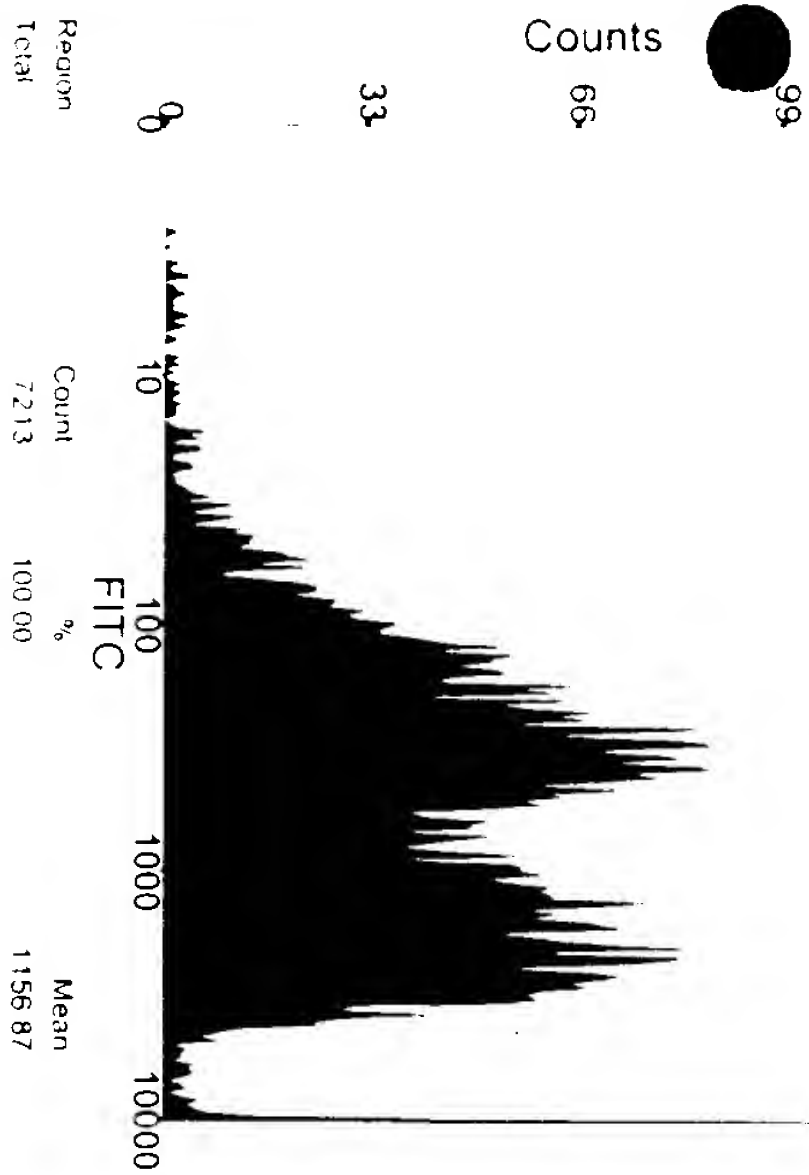


Region	Count	%	Mean
Total	9050	100.00	155.80, 1212.35
R4	7213	79.70	142.79, 1156.87



Region	Count	%	Mean
Total	7213	100.00	867.66, 1156.87
R2	1636	22.68	790.21, 103.34
R3	5467	75.79	888.59, 1304.66

133 G2: R4 & R1

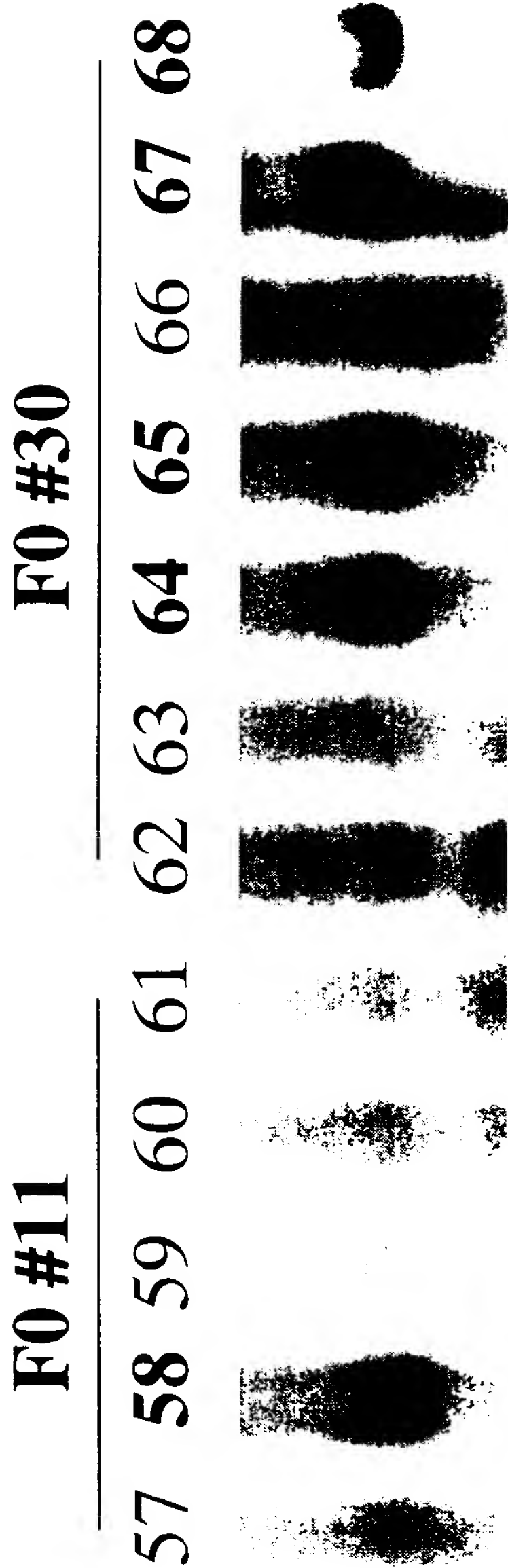


Region	Count	%	Mean
Total	7213	100.00	1156.87

Generation of Transgenic Mice from Two Different Linkers mAb
C and mAb D

Transgenic Mice Generated from mAb D Linker

by Southern Blot Analyses



Date: November 17, 2000

Transgenic Mice Generated from mAb C linker

by Southern Blot Analyses

F0 #46

30 32 33



Date: February 8, 2001